

THE JOURNAL
OF
CLINICAL INVESTIGATION

VOLUME XII
1933

PUBLISHED BY
THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

THE JOURNAL OF CLINICAL INVESTIGATION

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

Contents

Further Studies on the Agglutination Reaction in Chronic Arthritis	EDITH E. NICHOLLS AND WENDELL J. STAINSOY	505
Intestinal Studies II Pancreatic Enzymes in Pernicious Anemia	O. M. HELMER PAUL J. FOUTS AND L. G. ZEPFAS	519
Cramps A Clinical and Chemical Study	JOHN H. TALDOTT AND JOST MICHELSEN	533
Studies of Gastric Pepsin I Methods of Measurement and Factors which Influence It	ARNOLD E. OSTERBERG, FRANCES R. VANZANT AND WALTER C. ALVAREZ	551
Studies of Gastric Pepsin II Secretion of Pepsin in Cases of Duodenal Ulcer and Peptic Ulcer	FRANCES R. VANZANT, ARNOLD E. OSTERBERG, WALTER C. ALVAREZ AND ANDREW B. RIVERS	557
Studies of Urea Excretion VIII The Effects on the Urea Clearance of Changes in Protein and Salt Contents of the Diet	CUTHBERT L. COPE	567
Changes in Peripheral Vascular Disease During Sympathetic Ganglionectomy after General Anesthesia	WINCHELL MCK. CRAIG, BAYARD T. HORTON AND CHARLES H. ARD	573
Studies in the Blood of Patients with Pernicious Anemia	S. MILTON GOLDBRAMER	583
Protein Changes and Suspension Stability of the Blood in Lobar Pneumonia	ANNES K. MOEN AND HODART A. REIMANN	589
Studies in Cases of Unexplained Gastric Anacidity	W. SCOTT POLLARD	599

PUBLISHED BIMONTHLY

AT

PRINCE AND LEMON STREETS, LANCASTER, PA.

BY

THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

Made in United States America

The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

EDITORIAL BOARD

J HAROLD AUSTIN, M D, EDITOR-IN-CHIEF
University of Pennsylvania Medical School, Philadelphia, Pa

RANDOLPH WEST, M D, ASSISTANT EDITOR
Presbyterian Hospital, New York, N Y

ALFRED E COHN, M D, *New York, N Y*
WARFIELD T LONGCOPE, M D, *Baltimore, Md*
WILLIAM S McCANN, M D, *Rochester, N Y*

C PHILIP MILLER, JR, M D, *Chicago, Ill*
JOHN P PETERS, M D, *New Haven, Conn*
G CANBY ROBINSON, M D, *New York, N Y*

The Editorial Committee of the American Society for Clinical Investigation

HAROLD L AMOSS, M D, *Durham, N C*
HILDING BERGLUND, M D, *Minneapolis, Minn*
FRANCIS G BLAKE, M D, *New Haven, Conn*
ARTHUR L BLOOMFIELD, M D, *San Francisco, Calif*
C SIDNEY BURWELL, M D, *Nashville, Tenn*
HENRY A CHRISTIAN, M D, *Boston, Mass*
ROBERT F LOEB, M D, *New York, N Y*

WILLIAM S McCANN, M D, *Rochester, N Y*
W MCKIM MARRIOTT, M D, *St Louis, Mo*
EDWARD H MASON, M D, *Montreal, C*
THOMAS M RIVERS, M D, *New York, N Y*
LEONARD G ROWNTREE, M D, *Philadelphia, Pa*
FRED M SMITH, *Iowa City, Iowa*
ISAAC STARR, JR, M D, *Philadelphia, Pa*
JOSEPH T WEARN, M D, *Cleveland, Ohio*

THE JOURNAL OF CLINICAL INVESTIGATION is designed for the publication of original investigations dealing with or bearing on the problems of disease in man. Each volume will consist of 6 bimonthly issues appearing throughout the calendar year.

Contributions should be sent to the Editor-in-Chief, University of Pennsylvania Medical School, Philadelphia, Pa. They should ordinarily be limited in length to twenty printed pages, and papers exceeding this limit may be returned to authors for revision and reduction. Authors receive 50 reprints of their papers without covers free; additional reprints and covers may be purchased at standard prices. Further information regarding the preparation of manuscripts may be obtained from members of the Editorial Board of the Journal or of the Editorial Committee of The American Society for Clinical Investigation.

Beginning with Volume XI, January 1, 1932, the subscription price will be \$10 per volume of 6 bimonthly issues representing the issues of the calendar year and comprising a volume of about 1200 pages; foreign countries \$10.50. Volumes I to X inclusive are sold at \$5.00 per volume. Single copies sold when available. Subscriptions are net postpaid. Remittance should be made by draft, check or postal money order, payable to THE JOURNAL OF CLINICAL INVESTIGATION, Prince and Lemon Sts., Lancaster, Pa., or 654 Madison Ave., New York. Claims for copies lost in the mails must be received within thirty days (domestic), ninety days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

Correspondence concerning business matters should be addressed to THE JOURNAL OF CLINICAL INVESTIGATION, Prince and Lemon Sts., Lancaster, Pa., or 654 Madison Ave., New York.

THE JOURNAL OF CLINICAL INVESTIGATION

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

Contents

Studies on the Action of Diuretics II The Effect of Salrgan upon the Water Content of the Plasma as Measured by the Refractive Index HENRY LENZEN SCHMITZ	741
Studies in Congestive Heart Failure XVIII A Critical Study of Methods for Determining the Cardiac Output in Patients with Cardiac Disease. ARTHUR GROLLMAN BEN FRIEDMAN GURNEY CLARK AND F R HARRISON	751
Physiological Disturbances During Experimental Diphtheritic Intoxication I Blood Sugar Lactic Acid and Non Protein and Amino Acid Nitrogen HERMAN YANNET AND DANIEL C DARROW	767
Physiological Disturbances During Experimental Diphtheritic Intoxication II Hepatic Glycogenesis and Glycogen Concentration of Cardiac and Skeletal Muscle HERMAN YANNET AND DANIEL C DARROW	779
Physiological Disturbances During Experimental Diphtheritic Intoxication III Respiratory Quotients and Metabolic Rate. HERMAN YANNET AND WALTER GOLDFARB	787
Studies of Total Pulmonary Capacity and Its Subdivisions I Normal Absolute and Relative Values ALBERTO HURTADO AND CHARLES BOLLER	793
Studies of Total Pulmonary Capacity and Its Subdivisions II Correlation with Physical and Radiological Measurements ALBERTO HURTADO AND WALTER W FRAY	807
Studies of Total Pulmonary Capacity and Its Subdivisions III Changes with Body Posture ALBERTO HURTADO AND WALTER W FRAY	825
Studies of Total Pulmonary Capacity and Its Subdivisions IV Preliminary Observations on Cases of Pulmonary Emphysema and of Pneumoconiosis ALBERTO HURTADO WALTER W FRAY AND WM S MCCANN	833
The Effect of Digitalis on the Venous Pressure of Normal Individuals DAVID A RYTAND	847
Comparison of the Creatinine and Urea Clearance Tests of Kidney Function J M HAYMAN, JR. J A HALSTED AND L E SEYLER	861
Experiments on the Relation of Creatinine and Urea Clearance Tests of Kidney Function and the Number of Glomeruli in the Human Kidney Obtained at Autopsy J M HAYMAN, JR. AND S M JOHNSTON	877
Crum Treatment of Hemolytic Streptococcus Pneumonia HAROLD L AMOSS AND ERLF B CRAVEN JR.	885
Cystine Content of Finger Nails in Pellagra SHELDON A PAYNE AND WILLIAM A PIRLZWEIG	899
The Production of Experimental Plant Vincent's Angina in the Dog HELFNE WALLACE, E W WALLACE AND O H ROBERTSON	909
The Effects of Alternate Suction and Pressure on Blood Flow to the Lower Extremities EUGENE M. LANDIS AND JOHN H GIBBON JR.	925
Proceedings of the Twenty fifth Annual Meeting of the American Society for Clinical Investigation held in Washington, D C May 8 1933	963

PUBLISHED BIMONTHLY

AT

PRINCE AND LEMON STREETS, LANCASTER PA

BY

THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

Made in United States of America

The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

EDITORIAL BOARD

J HAROLD AUSTIN, M D, EDITOR-IN-CHIEF
University of Pennsylvania Medical School, Philadelphia, Pa

RANDOLPH WEST, M D, ASSISTANT EDITOR
Presbyterian Hospital, New York, N Y

ALFRED E COHN, M D, *New York, N Y*
WARFIELD T LONGCOPE, M D, *Baltimore, Md*
WILLIAM S McCANN, M D, *Rochester, N Y*

C PHILIP MILLER, JR., M D, *Chicago, Ill*
JOHN P PETERS, M D, *New Haven, Conn*
G CANBY ROBINSON, M D, *New York, N Y*

The Editorial Committee of the American Society for Clinical Investigation

HAROLD L AMOSS, M D, *Durham, N C*
HILDING BERGLUND, M D, *Minneapolis, Minn*
FRANCIS G BLAKE, M D, *New Haven, Conn*
ARTHUR L BLOOMFIELD, M D, *San Francisco, Calif*
C SIDNEY BURWELL, M D, *Nashville, Tenn*
HENRY A CHRISTIAN, M D, *Boston, Mass*
ROBERT F LOEB, M D, *New York, N Y*

WILLIAM S McCANN, M D, *Rochester, N Y*
W MCKIM MARRIOTT, M D, *St Louis, Mo*
EDWARD H MASON, M D, *Montreal, Can*
THOMAS M RIVERS, M D, *New York, N Y*
LEONARD G ROWNTREE, M D, *Philadelphia*
FRED M SMITH, *Iowa City, Iowa*
ISAAC STARR, JR., M D, *Philadelphia, Pa*
JOSEPH T WEARN, M D, *Cleveland, Ohio*

THE JOURNAL OF CLINICAL INVESTIGATION is designed for the publication of original investigations dealing with or bearing on the problems of disease in man. Each volume will consist of 6 bimonthly issues appearing throughout the calendar year.

Contributions should be sent to the Editor-in-Chief, University of Pennsylvania Medical School, Philadelphia, Pa. They should ordinarily be limited in length to twenty printed pages, and papers exceeding this limit may be returned to authors for revision and reduction. Authors receive 50 reprints of their papers without covers free, additional reprints and covers may be purchased at standard prices. Further information regarding the preparation of manuscripts may be obtained from members of the Editorial Board of the Journal or of the Editorial Committee of The American Society for Clinical Investigation.

Beginning with Volume XI, January 1, 1932, the subscription price will be \$10 per volume of 6 bimonthly issues representing the issues of the calendar year and comprising a volume of about 1200 pages, foreign countries \$10.50. Volumes I to X inclusive are sold at \$5.00 per volume. Single copies sold when available. Subscriptions are net postpaid. Remittance should be made by draft, check or postal money order, payable to THE JOURNAL OF CLINICAL INVESTIGATION, Prince and Lemon Sts., Lancaster, Pa., or 654 Madison Ave., New York. Claims for copies lost in the mails must be received within thirty days (domestic), ninety days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

Correspondence concerning business matters should be addressed to THE JOURNAL OF CLINICAL INVESTIGATION, Prince and Lemon Sts., Lancaster, Pa., or 654 Madison Ave., New York.

THE JOURNAL

OF

CLINICAL INVESTIGATION

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

Contents

The Diurnal Rhythm in Water and Mineral Exchange	ROBERT C. MANCHESTER	995
The Effect of Splenic Contraction on the Formed Elements of the Blood in a Case of Anemia and Splenomegaly	D. K. MILLER AND C. P. RHODES	1009
The Proteins of Blood and Subcutaneous Lymph in Dogs	A. A. WEECH, E. GOETTSCH AND E. B. REEVES	1021
Permeability of Capillaries to Plasma Lipoids	EVELYN B. MAN AND JOHN P. PETERS	1031
The Clinical Significance of the Erythrocytic Sedimentation Test in Rheumatoid Arthritis	WENDELL J. STAINSBY AND EDITH E. NICHOLLS	1041
The Oxygenation of Concentrated Versus Normal Bloods	GEORGE P. RAY, C. I. THOMAS AND J. E. STRONG	1051
Electrolytes in Human Tissue. III. A Comparison of Normal Hearts with Hearts Showing Congestive Heart Failure	WALTER E. WILKINS AND GLENN E. CULLEN	1063
The Cisternal Pressure in Congestive Heart Failure and Its Bearing on Orthopnea	WILLIAM GROCE HARRISON, JR.	1075
The Action of Phlorizin on the Excretion of Glucose, Xylose, Sucrose, Creatinine and Urea by Man	HERBERT CHASIS, NORMAN JOILIFF AND HOMER W. SMITH	1083
Amino Nitrogen Changes of the Blood in Nephritis	ESSEN KIRK	1091
A Formula and Nomogram for the Estimation of the Osmotic Pressure of Colloids from the Albumin and Total Protein Concentrations of Human Blood Sera	HERBERT S. WELLS, JOHN B. YOUNG AND DAVID G. MILLER, JR.	1103
Herpetic Pharyngitis and Stomatitis. A Report of Three Cases	PERRIN H. LONG	1119
Studies Upon the Nasal Secretions. I. The Cellular Content of the Nasal Secretions in Acute Disease of the Respiratory Tract	PERRIN H. LONG, FLEANOR A. BLISS AND HARRIETT M. CARPENTER	1127
Magnesium Metabolism in Hyperparathyroidism	HAROLD A. BULGER AND FLORENCE GAUSMANN	1135
Immunity in Diabetes. Influence of Diabetes on the Development of Antibacterial Properties in the Blood	RUSSELL RICHARDSON	1143
A Note on the Calculation of Water Exchange	L. H. NEWBURN, F. H. WILEY AND M. W. JOHNSTON	1151
The Rate of Excretion of Urine in Subjects with Different Amounts of Renal Tissue	DAVID A. RYTAND	1153
Salt and Water in the Treatment of Diabetic Acidosis	DAVID M. KIDD	1169
Index to Volume XII		1185

PUBLISHED BIMONTHLY

AT

PRINCE AND LEMON STREETS, LANCASTER, PA.

BY

THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

EDITORIAL BOARD

J HAROLD AUSTIN, M D, EDITOR-IN-CHIEF
University of Pennsylvania Medical School, Philadelphia, Pa

RANDOLPH WEST, M D, ASSISTANT EDITOR
Presbyterian Hospital, New York, N Y

ALFRED E COHN, M D, *New York, N Y*
WARFIELD T LONGCOPE, M D, *Baltimore, Md*
WILLIAM S McCANN, M D, *Rochester, N Y*

C PHILIP MILLER, JR, M D, *Chicago, Ill*
JOHN P PETERS, M D, *New Haven, Conn*
G CANBY ROBINSON, M D, *New York, N Y*

The Editorial Committee of the American Society for Clinical Investigation

HAROLD L AMOSS, M D, *Durham, N C*
HILDING BERGLUND, M D, *Minneapolis, Minn*
FRANCIS G BLAKE, M D, *New Haven, Conn*
ARTHUR L BLOOMFIELD, M D, *San Francisco, Calif*
C SIDNEY BURWELL, M D, *Nashville, Tenn*
HENRY A CHRISTIAN, M D, *Boston, Mass*
ROBERT F LOEB, M D, *New York, N Y*

WILLIAM S McCANN, M D, *Rochester, N Y*
W MCKIM MARRIOTT, M D, *St Louis, Mo*
EDWARD H MASON, M D, *Montreal, Can*
THOMAS M RIVERS, M D, *New York, N Y*
LEONARD G ROWNTREE, M D, *Philadelphia, Pa*
FRED M SMITH, *Iowa City, Iowa*
ISAAC STARR, JR, M D, *Philadelphia, Pa*
JOSEPH T WEARN, M D, *Cleveland, Ohio*

THE JOURNAL OF CLINICAL INVESTIGATION is designed for the publication of original investigations dealing with or bearing on the problems of disease in man. Each volume will consist of 6 bimonthly issues appearing throughout the calendar year.

Contributions should be sent to the Editor-in-Chief, University of Pennsylvania Medical School, Philadelphia, Pa. They should ordinarily be limited in length to twenty printed pages, and papers exceeding this limit may be returned to authors for revision and reduction. Authors receive 50 reprints of their papers without covers free, additional reprints and covers may be purchased at standard prices. Further information regarding the preparation of manuscripts may be obtained from members of the Editorial Board of the Journal or of the Editorial Committee of The American Society for Clinical Investigation.

Beginning with Volume XI, January 1, 1932, the subscription price will be \$10 per volume of 6 bimonthly issues representing the issues of the calendar year and comprising a volume of about 1200 pages, foreign countries \$10.50. Volumes I to X inclusive are sold at \$5.00 per volume. Single copies sold when available. Subscriptions are net postpaid. Remittance should be made by draft, check or postal money order, payable to THE JOURNAL OF CLINICAL INVESTIGATION, Prince and Lemon Sts, Lancaster, Pa., or 654 Madison Ave, New York. Claims for copies lost in the mails must be received within thirty days (domestic), ninety days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

Correspondence concerning business matters should be addressed to THE JOURNAL OF CLINICAL INVESTIGATION, Prince and Lemon Sts, Lancaster, Pa., or 654 Madison Ave, New York.

FURTHER STUDIES ON THE AGGLUTINATION REACTION IN CHRONIC ARTHRITIS¹

BY EDITH E. NICHOLLS AND WENDELL J. STAINSBY

*(From the Department of Medicine of Cornell University Medical College, the Second
(Cornell) Medical Division of Bellevue Hospital and the New York Hospital
New York City)*

(Received for publication November 30 1932)

The present authors (1), collaborating with Cecil, reported, in 1929, the isolation of streptococci from the blood and joints of patients having rheumatoid arthritis. A large percentage of the organisms thus recovered were culturally and biologically similar, and presented the appearance of hemolytic streptococci on blood agar plates. For convenience, these organisms were designated as "typical strains."

In later communications the authors reported, with Cecil (2), and alone (3, 4), that the serums of a high percentage of patients showing well developed signs of rheumatoid arthritis gave strong agglutination reactions with "typical strain" streptococci. The same serums gave positive agglutination results with a few hemolytic streptococci from sources other than arthritis, but whenever such reactions took place the organisms were culturally and biologically indistinguishable from "typical strain" streptococci. With other bacterial antigens, however, including indifferent and green producing streptococci, pneumococci, staphylococci and colon bacilli they gave little or no agglutination. Likewise, negative or insignificant reactions were obtained when the serums of a large number of normal individuals and of patients with other diseases were tested for agglutinations with "typical strain" streptococci. Among these control serums were 79 from rheumatic fever and 16 from osteoarthritis. As a result of this study, the authors concluded that the serums of a high percentage of patients with rheumatoid arthritis gave strong agglutination reactions with a biologically specific type of hemolytic streptococcus. Gray and Gowen (5), and Dawson, Olmstead and Boots (6) have in the main confirmed these observations.

THE PRESENT STUDY

In order to obtain further information concerning this phenomenon, routine agglutination tests were carried out with "typical strain" AB13 on the serums of all patients admitted to the arthritis department of the

¹ This study was carried on with the technical assistance of Edith L. Ross, Edna H. Lindsey, Edith M. Kirkpatrick, and Elnora B. Carmichael.

Cornell Clinic during a two-year period. These tests were repeated, when possible, at intervals throughout the patient's period of observation. The purpose of this paper is to present an analysis of the findings in this study. Unless otherwise stated, all agglutination results reported refer to those at the time of the patient's first visit to the Clinic.

Seven hundred and thirty-three cases are included—a few had to be discarded either because a diagnosis could not be made or because the patients were suffering from diseases other than arthritis. The incidence of each disease is given in Table 1.

TABLE 1
Tabulation of diseases studied

Disease	Number of cases
Rheumatoid arthritis	613
Osteoarthritis	44
Monarticular arthritis	41
Rheumatic fever	14
Gonococcus arthritis	16
Gout	4
Intermittent hydrarthrosis	1
Total	733

AGGLUTINATION TECHNIC

The method of performing the agglutination test was similar to that previously described (3) and so need not be explained in detail. No dilutions were made beyond 1:5120. Each test was controlled with a serum that was known to possess no streptococcus agglutinins and with a tube containing 0.5 cc of broth and 0.5 cc of culture. All agglutinations were heated in a water bath at 56° C for two hours. The tubes were then placed in a refrigerator and readings were made the following morning. The last dilution in which definite clumping of the bacteria could be detected by the naked eye was recorded as the agglutination titer.

The agglutination tests on each specimen were carried out on two consecutive days and if the readings thus obtained did not correspond, the procedure was repeated until the titer was accurately ascertained.

Occasionally the antigen showed a tendency to become granular. This proclivity was usually overcome by daily transferring the culture to fresh blood broth mediums over a period of several days. Subculturing in broth containing 0.2 per cent disodium hydrogen phosphate, in place of the 1.5 sodium chloride, was also found to encourage diffuse growth of the organisms.

RHEUMATOID ARTHRITIS

Six hundred and thirteen patients were diagnosed as suffering from rheumatoid arthritis—none were included in this group who had symptoms in one joint only.

For convenience, the patients were allotted to one of three groups, according to the degree of joint involvement. The first group includes those who complained of pain in the joints, but failed to show any periarticular swelling, the symptoms were usually migratory in character and tenderness was frequently found. The second group is comprised of those who presented the usual picture of rheumatoid arthritis with characteristic periarticular swelling, generally including fusiform fingers. The third group is similar to the second, but is made up of patients who were in a more advanced stage of the disease—deformities such as ulnar deviation, and partial or complete ankylosis of one or more joints characterize this group. These three groups are indicated by +, ++, and +++, respectively, and the number and percentage of patients in each group are shown in Table 2.

TABLE 2
Tabulation of patients with rheumatoid arthritis

Group	Number of patients	Per cent
+	310	50.6
++	241	39.3
+++	62	10.1
Total	613	100.0

In several tables and figures reference is made to mean agglutination titers. These group averages are computed by multiplying each figure representing a dilution of serum by the number of serums agglutinating at that titer, adding these products and dividing the sum by the total number of serums included in the group.

In order that insignificant information be not included in the tables and figures, titers of 1:20 and 1:40 have been recorded as negative.

Relation of incidence of disease to age of patient

In Figure 1 the age incidence of patients belonging to each of the three groups (+, ++, +++) is indicated. Only one patient under ten years of age was seen with rheumatoid arthritis, and only seven, in the second decade of life, while there was a sharp falling off after sixty years of age. That rheumatoid arthritis is chiefly a disease of middle life is clearly shown in Figure 1. The small proportion over 60 years of age may be due in part to the failure of patients at this age to seek medical advice, but no such reasoning is applicable to the low incidence in patients of twenty years of age or under. It must, therefore, be concluded that rheumatoid arthritis is a relatively rare disease during the first two decades of life. While the three curves are nearly parallel, there is a distinct tendency in the + group to mark its maximum incidence at an earlier age than do the others.

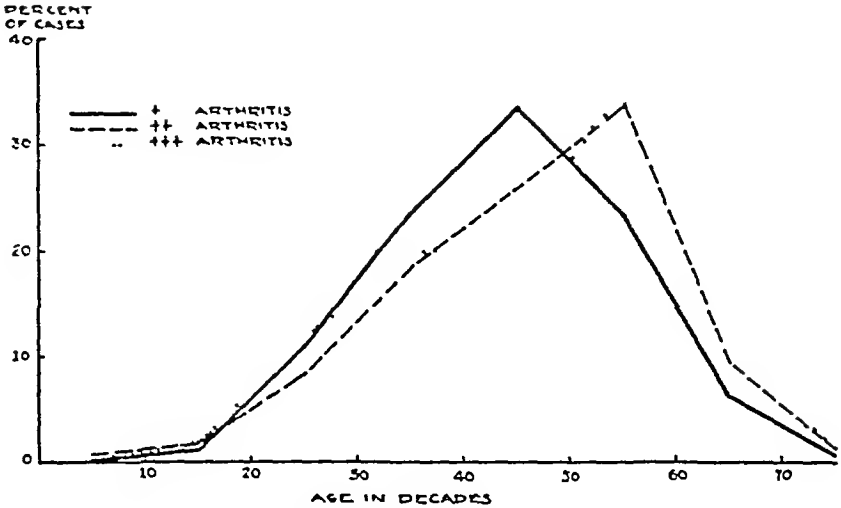


FIG 1 RELATIONSHIP OF INCIDENCE OF DISEASE TO AGE OF PATIENTS IN RHEUMATOID ARTHRITIS (613 CASES)

Relation of agglutination titer to degree of joint involvement

In a previous study of agglutination reactions with rheumatoid arthritis serums a selection of patients with marked joint involvement was used. Of the 110 serums then examined, 93.6 per cent showed definite agglutination to a titer of 1:640 or more (3). In the present study of 613 cases all stages of rheumatoid arthritis are represented. In Figure 2

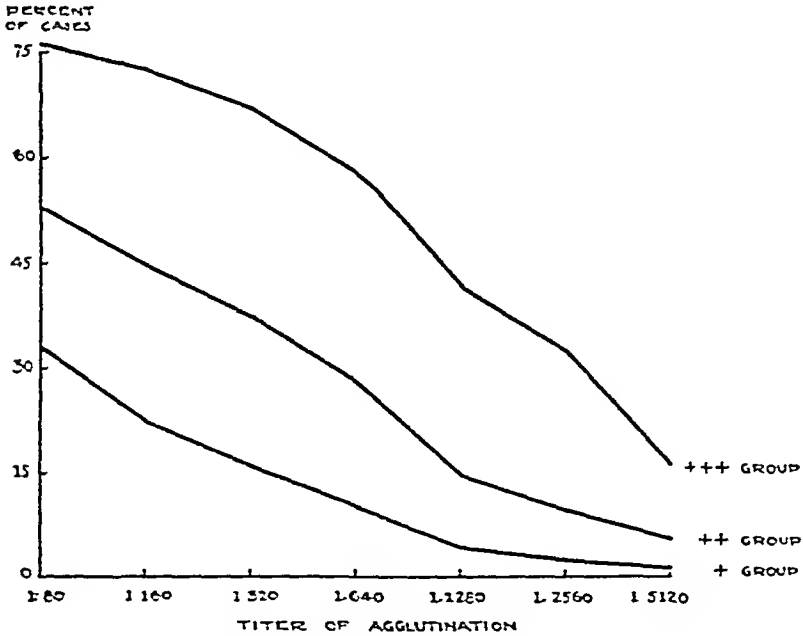


FIG 2 RELATION OF DEGREE OF JOINT INVOLVEMENT TO TOTAL AGGLUTINATION (613 CASES)

is indicated the total agglutinations for each of the three groups. The highest degree of agglutination was found in the class with advanced rheumatoid arthritis (+++), while a distinctly higher agglutination was found in the moderately advanced group than in the mild cases without swelling (+). From these results it is evident that, on an average, the more advanced the joint involvement, the higher the titer of agglutination.

Relation of agglutination titer to duration of disease

The agglutination titer in rheumatoid arthritis with relation to the duration of the disease is indicated in Table 3 and Figure 3. In 8 cases of disease duration of one month or less there was not the slightest evidence of the presence of agglutinins. In 45 cases of disease duration

TABLE 3

Results of agglutination tests in rheumatoid arthritis in relation to duration of disease

Duration	Cases	Neg ative	Titer of agglutination							Mean titer
			1 80	1 160	1 320	1 640	1 1280	1,2560	1,5120	
One month or under	8	8								0
Over 1 month to 3 months	45	30	6	5	2	1			1	1 171
Over 3 months to 6 months	66	31	5	6	5	11	3	2	3	1 520
Over 6 months to 1 year	92	49	14	4	9	8	1	3	4	1 426
Over 1 year to 3 years	126	62	10	5	12	16	8	7	6	1 591
Over 3 years to 5 years	88	42	11	8	5	9	6	4	3	1 486
Over 5 years	188	115	7	13	13	17	7	7	9	1 482

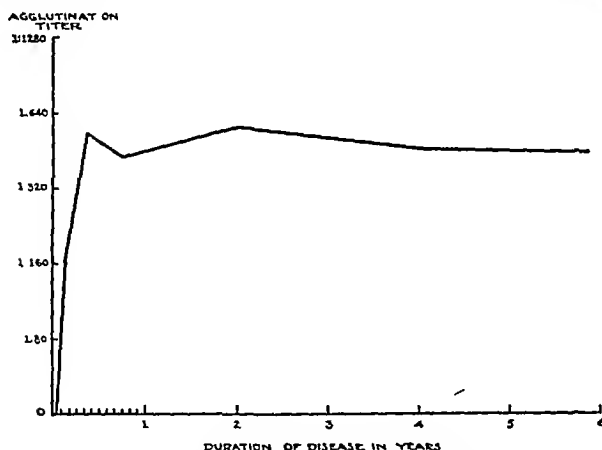


FIG 3 MEAN AGGLUTINATION TITER IN RHEUMATOID ARTHRITIS IN RELATION TO DURATION OF DISEASE (613 CASES)

from one to three months the highest agglutination titer recorded with one exception was 1 640 and the mean was 1 171. In the group having the disease from three to six months, 3 showed an agglutination titer of 1 5120, while the average was 1 520. In the groups covering a disease duration of over six months little variation was noted, the line on the graph closely approaching a straight one. A similar analysis, employing only cases in groups ++ and +++, revealed a curve similar to that when all three groups were used.

From these results it is evident that it takes six months, on the average, for a patient to develop hemolytic streptococcus agglutinins to the maximum titer, but that aside from this initial period the duration of the disease plays no important part in the strength of the agglutination reaction. Dawson, Olmstead and Boots (6), reporting a series of 153 cases, stated that the agglutination titer increased with the duration of the disease. Our findings do not confirm this conclusion.

Relation of agglutination titer to age of patient

In Table 4 and Figure 4 the results of the agglutination tests in rheumatoid arthritis are arranged in relation to the age of the patients. A boy of seven with moderately advanced rheumatoid arthritis was the only patient under 10 years of age seen in the arthritis clinic. His serum

TABLE 4

Results of agglutination tests in rheumatoid arthritis in relation to age of patient

Age	Cases	Neg ative	Titer of agglutination							Mean titer
			1 80	1 160	1 320	1 640	1 1280	1 2560	1 5120	
<i>Years</i>										
Under 10	1					1				
10 to 19	7	4	1			2				1 194
20 to 29	62	34	8	3	4	4	2	2	5	1 616
30 to 39	129	64	18	9	9	9	9	7	4	1 476
40 to 49	184	108	10	12	16	21	3	5	9	1 457
50 to 59	174	94	12	15	13	19	9	6	6	1 444
60 to 69	50	30	4	2	3	5	2	3	1	1 403
70 or over	6	3			1	1	1			

showed an agglutination titer of 1 640 with "typical strain" streptococcus AB13. There were but 7 patients between ten and nineteen years of age—serums from 2 of these gave an agglutination titer of 1 640, from 1 a titer of 1 80, and from 4 there were negative findings, with a mean agglutination titer for the group of 1 194. The age period covering the third decade gave, on an average, the highest titer of agglutination, while in the periods following there was a slight gradual drop all the way to the last group which included patients seventy years of age or older.

Dawson, Olmstead and Boots (6) reporting a sharply progressive increase in the median agglutination titers of patients in the third, fourth and fifth decades, concluded that "the property of rheumatoid arthritis serum responsible for the agglutination of hemolytic streptococci is definitely related to the age of the patient." Our results do not agree with their findings. When, however, groups +, ++ and +++ of our series are plotted individually, group +++ tends to show increased titers of agglutination in the fourth, fifth and sixth decades. It seems probable then that the series of cases reported by Dawson, Olmstead and Boots is preponderantly made up of cases corresponding to those in group +++ of the present series. In the opinion of the writers, a more accurate impression of the relation of age to the titer of agglutination can be obtained from the study of the disease as a whole than from an analysis of certain of its phases.

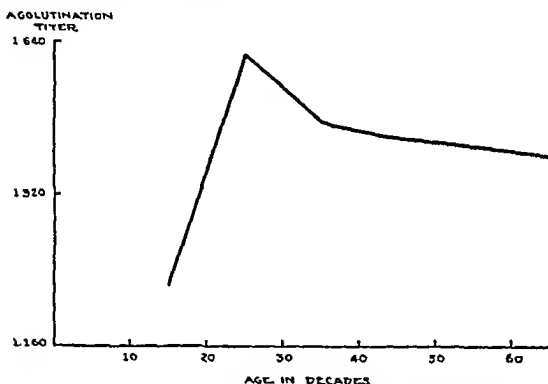


FIG 4 MEAN AGGLUTINATION TITER IN RHEUMATOID ARTHRITIS IN RELATION TO AGE OF PATIENT (606 CASES BETWEEN THE AGES OF 10 AND 69)

In the present series, the number of patients in the first, second and eighth decades of life is too small to draw any definite conclusions concerning their agglutination reactions with "typical strain" streptococci. It seems probable, however, that in patients under twenty years of age the agglutination titer averages lower than in patients over that age. This impression receives support by the findings in a study of agglutinins in the serums of six patients in Bellevue Hospital suffering from Still's disease, a condition accepted by many as rheumatoid arthritis in early childhood. Although the same technic was carried out, negative results were obtained in each case. It is doubtful if a lower titer in patients under twenty years of age has any significance because during that period rheumat

thritis is uncommon and when it does occur it assumes atypical forms, frequently resembling rheumatic fever

In summing up the relationship of the age of the patient to the agglutination titer, one is led to the conclusion that with the possible exception of the first two decades of life the age of the patient plays no important role in the strength of the agglutination reaction

Relation of agglutination titer to time of year

Clinically, it is generally recognized that in temperate climates patients with rheumatoid arthritis are usually at their worst in cold weather and at their best in hot weather. In order to ascertain if there is any seasonal variation in the agglutination titers in this disease Table 5 was prepared

TABLE 5

*Results * of agglutination tests in rheumatoid arthritis in relation to season of year*

Season	Cases	Neg ative	Titer of agglutination							Mean titer
			1 80	1 160	1 320	1 640	1 1280	1 2560	1 5120	
Winter	98	33	12	8	9	17	8	7	4	1 660
Spring	79	30	3	4	7	16	3	5	11	1 1074
Summer	46	30	3	2	1	4	1	2	3	1 548
Autumn	80	35	4	6	12	7	4	7	5	1 728

* Total of 303 cases—groups ++ and +++ only

Cases of arthritis in groups ++ and +++ were selected as patients in the more advanced stages appeared to show the greatest seasonal variation. In the table, "Winter" is used to designate the period from January 1 to March 31, "Spring," from April 1 to June 30, "Summer," from July 1 to September 30, and "Autumn," from October 1 to December 31. The findings are interesting. The mean agglutination titer of 79 patients studied in the spring was 1 1074, while that of 46 studied in the summer was only 1 548. The mean titers during the autumn and winter were 1 728 and 1 660 respectively. Due to the small number of cases in this series and the distribution of the agglutination titers entering into the means, the degree of probability of chance occurrence of the differences between the means is too high to warrant any definite conclusions. Nevertheless the results are at least suggestive that on the average the agglutination titers are higher in the spring and lower in the summer than at other seasons of the year.

Relation of titer of agglutination to clinical changes

Initial agglutination tests on all patients were repeated at irregular intervals throughout each period of observation. Condensed protocols of those subjected to the test four or more times appear in Table 6. The

TABLE 6
Patients with repeated agglutinations

Pa- tient num- ber	Sex	Age	Dura- tion of disease	Joint in- volve- ment	Results of agglutinations										Clinical condition during observation		
					Date	Titer	Date	Titer	Date	Titer	Date	Titer	Date	Titer			
1	M	4	1 year	++	12/29/30	1:510	1/13/31	1:510	2/2/31	1:510	2/2/31	1:510	4/9/31	1:510	5/19/31	1:510	Unchanged
2	F	40	3 years	++	4/16/30	1:510	2/1/31	1:280	4/19/31	1:250	4/23/31	1:510	5/9/31	1:510	5/23/31	1:510	Gradually worse
3	F	26	6 months	++	1/20/30	1:640	3/1/31	1:610	4/29/31	1:250	1/1/32	1:250	2/1/32	1:250	2/23/32	1:250	Unchanged
4	M	23	2 years	++	4/15/31	1:640	3/6/32	1:30	10/9/31	1:250	1/1/32	1:320	2/1/32	1:640	2/1/32	1:640	Unchanged
5	P	43	8 years	++	1/18/31	1:640	4/9/31	1:30	6/29/31	1:560	10/16/31	1:640	1/1/32	1:640	1/1/32	1:640	Unchanged
6	P	48	0 months	++	1/18/31	1:640	4/13/31	1:510	6/29/31	1:560	10/9/31	1:560	1/1/32	1:560	1/1/32	1:560	Unchanged
7	F	57	2 months	++	2/7/31	1:160	6/10/31	1:80	9/30/31	1:320	1/6/32	1:320	1/6/32	1:320	1/6/32	1:320	Unchanged
8	P	88	15 years	++	10/9/31	1:2560	6/23/31	1:320	3/30/32	1:510	6/1/32	1:320	6/1/32	1:320	6/1/32	1:320	Unchanged
9	M	63	5 years	++	1/20/31	1:640	10/7/31	1:80	10/14/31	1:80	1/16/32	1:640	4/13/32	1:30	4/13/32	1:30	Probably slightly worse
10	P	50	3 years	++	10/6/31	1:30	1/6/32	1:510	2/4/32	1:250	4/13/32	1:250	5/23/32	1:30	5/23/32	1:30	Unchanged
11	F	50	3 months	++	8/2/31	neg.	10/2/31	1:30	10/23/31	1:510	4/8/32	neg.	5/13/32	neg.	5/13/32	neg.	Unchanged
12	F	48	6 years	++	4/17/31	1:610	9/18/31	1:30	11/27/31	1:160	3/4/32	1:160	3/4/32	1:160	3/4/32	1:160	Unchanged
13	F	33	4 months	++	2/15/31	1:2560	8/6/31	1:560	6/23/31	1:510	1/1/32	1:510	1/1/32	1:510	1/1/32	1:510	Gradually worse
14	F	30	14 years	++	5/16/31	1:560	10/23/31	1:560	6/23/31	1:510	1/1/32	1:510	1/1/32	1:510	1/1/32	1:510	Unchanged
15	M	20	3 years	++	1/18/31	neg.	5/1/31	1:30	10/30/31	1:320	3/30/32	1:640	3/30/32	1:640	3/30/32	1:640	Irregular course. In general unchanged
16	M	58	4 years	++	4/22/31	1:510	8/14/31	1:160	10/16/31	1:640	2/2/32	1:30	2/2/32	1:30	2/2/32	1:30	Irregular but definite improvement
17	P	31	7 years	++	12/10/31	1:960	4/1/31	1:610	6/17/31	1:30	4/13/32	neg.	4/13/32	neg.	4/13/32	neg.	Slight improvement
18	M	41	3 years	++	12/11/30	1:640	2/1/31	1:30	4/20/31	1:40	0/16/31	1:80	0/16/31	1:80	0/16/31	1:80	Gradually improved
19	M	4	10 weeks	++	12/20/30	neg.	1/21/31	1:640	1/13/31	1:1260	2/7/31	1:80	2/7/31	1:80	2/7/31	1:80	Discharged cured
20	M	9	4 months	++	4/11/30	1:640	1/22/30	1:640	3/3/31	1:160	6/23/31	neg.	6/23/31	neg.	6/23/31	neg.	Discharged cured
21	F	37	3 months	++	7/10/31	1:160	1/25/31	1:30	1/20/31	neg.	2/16/31	1:320	2/16/31	1:320	2/16/31	1:320	Gradually improved
22	F	28	6 months	++	4/8/31	1:660	4/26/31	1:560	6/23/31	1:640	10/29/31	1:320	10/29/31	1:320	10/29/31	1:320	Gradually improved
23	P	38	3 years	++	2/11/31	1:80	4/1/31	1:1260	8/9/31	1:30	1/13/32		1/13/32		1/13/32		Cured (4/15/32)
24	F	31	4 years	++	1/18/31	1:280	4/2/31	1:510	10/16/31	neg.	1/7/32	neg.	1/7/32	neg.	1/7/32	neg.	Constant improvement beginning spring 1931
25	F	34	3 years	++	7/22/31	1:1780	10/4/31	1:510	10/23/31	neg.	1/4/32	neg.	1/4/32	neg.	1/4/32	neg.	Constant improvement beginning spring 1931
26	F	30	3 years	++	12/10/30	1:80	3/1/31	1:640	6/23/31	neg.	1/4/32	neg.	1/4/32	neg.	1/4/32	neg.	Marked improvement beginning spring 1932
27	M	32	2 years	++	5/30/31	1:560	1/7/32	1:640	3/30/32	1:640	4/29/32	neg.	4/29/32	neg.	4/29/32	neg.	Marked improvement beginning spring 1932
28	P	35	2 years	++	1/18/31	1:1280	3/4/31	1:610	6/29/31	1:180	4/8/32	1:320	4/8/32	1:320	4/8/32	1:320	Irregular course. Improved last 6 months
29	F	30	1 year	+	1/10/30	1:80	6/10/31	1:280	5/16/32	1:80	4/6/32	1:160	4/6/32	1:160	4/6/32	1:160	Improvement beginning summer 1932
30	M	40	4 months	+	1/7/31	1:640	6/2/31	1:510	0/9/31	1:250	4/6/32	1:160	4/6/32	1:160	4/6/32	1:160	Severe exacerbations during spring of 1931 4/16/32 condition about as on admission Worse during first 6 months, 4/6/32 much im- proved

findings on one patient who had but three examinations were included because of their special interest

Patients 1 to 15, inclusive, showed no appreciable change in clinical condition throughout the observation period. In these cases the titers of agglutination evidenced a strong tendency to remain at a constant level.

In contrast to the findings on the fifteen patients mentioned are those of patients 16 to 28 who showed definite clinical improvement, sometimes with complete recovery. In this group the serums tended either to agglutinate at lower levels or else to completely disappear, but generally the strength of the agglutination did not show signs of diminishing until some weeks or months after the patient began to improve clinically. Patient 19 illustrates this proclivity very well. This patient with a history of polyarthritis of only ten weeks standing, whose illness was running a subacute course, presented the typical features of rheumatoid arthritis at the time of his first visit. An agglutination test at that time (December 20, 1930) gave negative results. The patient showed constant improvement dating from the time of his first visit and was entirely free from signs and symptoms on January 13, 1931. In spite of this clinical improvement, the agglutination titer continued to rise for one month and only began to fall after the patient was entirely free from signs and symptoms of the disease.

Patient 23, with a more chronic form of the disease, illustrates the same process. With a history of rheumatoid arthritis of three years duration she showed marked improvement from the Spring of 1931, yet it was several months before the agglutination titer began to diminish and not until one year later that the agglutinins disappeared completely.

Patients 29 and 30 illustrate the tendency of the agglutination titers to rise and fall with changes in the clinical condition. At their first visit both of these patients presented the picture of a very mild and early rheumatoid arthritis. While under treatment their conditions gradually became very much worse, with a definite increase in the agglutinin content of their serums. At a still later period the arthritis showed definite abatement, which condition was followed by a diminution of streptococcus agglutinins in the serums, evidenced by a lower agglutination titer.

In summing up the results of repeated agglutination reactions in rheumatoid arthritis, it is apparent that the agglutinin content in the patient's serum is related to his clinical condition, tending to increase gradually during the development of the disease, and to decrease gradually with the recovery of the patient. There seems also to be considerable individual variation in the time required for clinical change to be manifested in the agglutination reactions.

OTHER FORMS OF ARTHRITIS

The results of agglutination tests in other forms of arthritis seen during the period of this study are presented in Table 7.

TABLE 7

Results of agglutination tests in other forms of arthritis

Disease	Neg ative	Titer of agglutination							Cases
		1 80	1 160	1 320	1 640	1 1280	1 2560	1 5120	
Osteoarthritis	38	6							44
Monarticular arthritis	33	4	1	2	1				41
Rheumatic fever	11	1	1	1					14
Gonococcus arthritis	14	2							16
Gout	4								4
Intermittent hydrarthrosis	1								1

Forty four patients were diagnosed as suffering from osteoarthritis—special care being taken not to confuse true osteoarthritis with rheumatoid arthritis showing secondary osteoarthritic changes. All of these patients were over forty years of age and not one serum of the 44 gave an agglutination reaction to a titer higher than 1 80, while 38 were completely negative.

In the group classified as monarticular arthritis are included, for convenience, cases of spondylitis. Probably some of these 41 cases were of infectious origin of the rheumatoid arthritis type, but because of the uncertainty of diagnosis they were segregated in this way, 33 gave negative results to agglutination tests, while the highest titer recorded by the remaining 8 was 1 640.

Fourteen patients suffered from rheumatic fever. Eleven gave negative agglutinations, while the highest titer recorded was 1 320.

Sixteen cases of gonococcus arthritis were seen. A diagnosis made from the history of the association of gonococcus urethritis with the onset of joint symptoms was frequently confirmed by positive findings in a complement fixation test. The highest agglutination recorded for this group was at a titer of 1 80.

Four cases of gout and one of intermittent hydrarthrosis gave completely negative reactions.

SUMMARY AND DISCUSSION

Seven hundred and thirty three patients with chronic arthritis were studied by agglutination tests with "typical strain" streptococcus AB13.

In this aggregation rheumatoid arthritis was the predominating disease, as 613 of the patients were suffering from that affection. The age incidence in this group was an interesting feature. Only one patient under ten years of age was seen and there was a gradual increase up to and including the sixth decade, following which there was a sharp drop. The maximum incidence of patients without joint swelling occurred at a somewhat earlier age than the highest number with joint swelling.

A previous article by the present authors showed that the serums of a high percentage of selected patients with well-developed rheumatoid arthritis gave strong agglutination reactions with a specific type of hemolytic streptococcus. In the present study, all types of rheumatoid arthritis are included and a more detailed analysis of the results has been made.

Apparently, the degree of joint involvement is an important factor in the strength of the agglutination reaction, as the tests proved that the more marked the joint involvement, the higher the agglutination titer. Moreover, as the amount of joint involvement is generally considered to be commensurate with the severity of the disease, it does not seem unfair to assume that as the arthritis increases in severity more evidence is found of streptococcus agglutinins in the serum.

An analysis of the agglutination reaction in relation to the duration of the disease reveals the fact that it takes several weeks following the onset of the disease for the streptococcus agglutinins to become apparent in the serum, and that the maximum titer is not obtained until an average of six months has elapsed. Except in this relation, the duration of the disease appears to play no important role in the agglutination phenomenon.

The age of the patient, likewise, showed little effect on the strength of the agglutination reaction. An exception must be made, however, in the first two decades of life. Although one patient seven years of age gave a definite agglutination to a titer of 1:640, the tendency was for the titer in subjects of this age to be weaker and more irregular. As has already been pointed out, little significance can be attached to this observation because such patients seldom manifest the typical clinical picture of rheumatoid arthritis, their signs and symptoms frequently resembling those of rheumatic fever, a disease that does not give positive results in tests with "typical strain" streptococci.

Of particular significance are the findings in patients subjected to repeated agglutination tests extending over a period of months or years. The results of these tests are remarkably consistent as the serums of patients in whom the disease appears to be stationary continue to give similar agglutination titers throughout the period of observation. In patients fortunate enough to show marked improvement or complete recovery, on the other hand, the streptococcus agglutinins either diminish or completely disappear from the blood. Sometimes this is accomplished in a few weeks, sometimes it takes months or even years.

With the information at hand concerning the agglutination reaction in rheumatoid arthritis, it would seem justifiable to consider the nature of this phenomenon. Unfortunately, we do not have a chronic disease that affords a comparison and must, perforce, compare it with an acute disease, such as typhoid fever. In typhoid the Widal reaction is considered to be an immunological one. Following the onset of the disease,

there is a definite interval of several days before a positive typhoid agglutination can be obtained. In the succeeding days or weeks the strength of the reaction tends to increase, while on recovery of the patient it slowly diminishes and in the course of several months or years eventually disappears. In rheumatoid arthritis a similar situation exists. The only essential factor in which it differs from that in typhoid fever is the time element. In typhoid the agglutination changes are apparent after periods of days—in arthritis, after periods of weeks or months. This would seem to be a natural disparity between the processes in an acute and a chronic disease. Although not irrefutably proven, these findings afford strong evidence that the presence of streptococcus agglutinins in such high titers is a true immunological response to a bacterial invader similar in all essential respects to that seen in typhoid fever.

In contrast to the positive results obtained from agglutination tests in rheumatoid arthritis are the negative results in osteoarthritis, gonococcus arthritis, rheumatic fever, and gout. A knowledge of this reaction is of practical value in differential diagnosis. A positive reaction at titers of 1:320 or higher is indicative of rheumatoid arthritis but the reverse does not hold true because a small percentage of patients even though they present the typical picture of rheumatoid arthritis have serums that give negative agglutination results with "typical strain" streptococci.

CONCLUSIONS

- 1 A high percentage of patients with rheumatoid arthritis give positive agglutination reactions to a specific type of hemolytic streptococcus.
- 2 In patients with advanced joint involvement higher average titers are obtained than in those with less involvement.
- 3 The duration of the disease and the age of the patient play unimportant roles in the strength of the agglutination.
- 4 Following the onset of the disease, there is a gradual increase in the agglutination titer which reaches its maximum in 6 months, on the average, while following recovery of the patient the agglutinins tend to diminish and eventually to disappear.
- 5 This reaction of serum in rheumatoid arthritis appears to be a true immunological response.
- 6 Other forms of arthritis do not give positive results in agglutination tests with typical strain streptococci.

BIBLIOGRAPHY

- 1 Cecil, R. L., Nicholls, E. E., and Stainsby, W. J. *Arch. Int. Med.* 1929, **xliv**, 571. The Bacteriology of the Blood and Joints in Chronic Infectious Arthritis.
- 2 Cecil, R. L., Nicholls, E. E., and Stainsby, W. J. *Am. J. M. Sc.*, 1931, **clxxi**, 12. The Etiology of Rheumatoid Arthritis.

- 3 Nicholls, E E , and Stainsby, W J , J Clin Invest , 1931, 1, 323 Streptococcal Agglutinins in Chronic Infectious Arthritis
- 4 Nicholls, E E , and Stainsby, W J , J A M A , 1931, 107, 1146 Streptococcal Agglutinins in Rheumatoid Arthritis
- 5 Gray, J W , and Gowen, C H , Am J M Sc , 1931, 11, 682 The Rôle of the Streptococcus in Arthritis Deformans
- 6 Dawson, M H , Olmstead, M , and Boots, R H , J Immunol , 1932, 1, 187 and 205 Agglutination Reactions in Rheumatoid Arthritis I Agglutination Reactions with Streptococcus Hemolyticus II The Nature and Significance of Agglutination Reactions with Streptococcus Hemolyticus

GASTRO-INTESTINAL STUDIES II PANCREATIC ENZYMES IN PERNICIOUS ANEMIA

By O M HELMER, PAUL J FOUTS AND L G ZERFAS

*(From the Lilly Laboratory for Clinical Research, Indianapolis City Hospital and the
Department of Medicine, Indiana University School of Medicine, Indianapolis)*

(Received for publication December 5 1932)

In a previous article (1) an attempt was made to correlate the findings in the gastric juice of patients having pernicious anemia with the clinical condition of the patients, the degree of central nervous system involvement present, and the maintenance dose of liver extract. Little or no correlation could be found, therefore, this study of the duodenal contents of patients having pernicious anemia was undertaken. The work of Castle and his associates (2) also indicated that additional studies on the digestion of proteins in cases of pernicious anemia should be carried out.

Ehrmann and Lederer (3) (1908) were the first to describe the results of duodenal analyses in cases of achylia gastrica, normal or slightly higher values being found by them. Einhorn (4) (1910) reported the results of the examination of 7 cases of achylia gastrica, in which 4 had normal trypsin values, 1 a low value, and 2 a complete absence of trypsin. In 1914 he reported (5) two cases of achylia gastrica with chronic pancreatitis in which there was an absence of trypsin but in which the other duodenal enzymes were approximately normal. He found (6) high enzymatic activity in the duodenal contents of 3 other cases with achylia gastrica, in 1918.

Crohn (7), in 1913, recorded a case of gastric achylia in which there were normal amounts of all duodenal enzymes except pancreatic rennin. In 1915, he (8) stated that "in my present series, consisting of over 120 cases, 103 cases are regarded as presenting normal pancreatic function, these include many pathological conditions, gastric lesions, benign and malignant, achylia gastrica, organic syphilis, exophthalmic goitre, secondary and primary anemias, malignant growths of various organs, diabetes mellitus, etc." Chace and Myers (9), in 1913, reported 1 case of pernicious anemia and 3 other cases of achylia gastrica in which there were normal amounts of duodenal enzymes. White (10), in 1916, studied another case of pernicious anemia and 3 other cases of achylia gastrica, all four having normal or high pancreatic enzyme values. In 1922, McClure and Jones (11), after studying two cases of pernicious anemia in severe relapse and two other cases of achylia gastrica, concluded that "in achylia gastrica and pernicious anemia no abnormalities in activity of

the external secretory function of the pancreas were demonstrable, as measured by the enzyme determination of the duodenal contents " In 1922, Roth and Sternberg (12) reported 6 cases of achylia gastrica in which the pancreatic enzymes were present Kahn (13) also, in 1923, described one case of sprue with gastric and pancreatic achylia The pancreatic achylia disappeared on proper diet, although the gastric achylia persisted McClure, Montague, and Mortimer (14), in 1924, found another case of achylia gastrica in which the pancreas functioned normally both as to enzymes and alkaline fluid Silverman and Denis (15), in 1924, observed one case of achylia gastrica with approximately normal duodenal enzymes Piersol and Bockus (16) recorded the findings in 3 cases of achylia gastrica in 1925 In two of these cases there was a rather low trypsin activity, while, in the third, trypsin was absent in one examination and present in a higher percentage than normal in another Landau et al (17), in 1926, reported 4 cases of pernicious anemia in which there was both gastric and pancreatic achylia By 1929 Landau and Glass (18) had collected 9 cases of pernicious anemia with both gastric and pancreatic achylia (Four of these had been reported in their previous article) They stated that, since the introduction of duodenal and gastric analyses, they had done daily examinations and these nine cases were the only ones in which both gastric and pancreatic achylia was found

Martin (19), in 1927, found normal amounts of trypsin and diastase in the duodenal contents of two cases of achylia gastrica Okada et al (20), in 1929, after studying the pancreatic enzymes of 7 cases of achylia gastrica stated that "evidence that the pancreatic secretion was disturbed as the consequence of disturbed gastric secretion was not found " Cheney and Niemand (21), in 1932, stated that fasting gastric contents contain approximately the same concentration of pancreatic enzymes as the fasting duodenal contents They made determinations of trypsin on the fasting gastric contents of 10 cases of pernicious anemia in relapse and in 9 of these there was an absence of trypsin, while, in the other, only a small amount of trypsin was found In 60 other cases (mainly achylia) normal amounts of trypsin were found in the fasting gastric contents

In the series of experiments reported in this paper, we have attempted not only to evaluate the enzymatic activity of the pancreas, but to determine, as well, the ability of the duodenum to activate the trypsinogen secreted by the pancreas To do this we have ascertained the tryptic power of the duodenal contents before and after incubation with enterokinase prepared from the duodenal mucosa of hogs

MATERIAL AND METHODS

Five young, healthy adults without evidence of disease and having had previous normal gastric analyses were used as controls All of the 22 cases of

pernicious anemia were typical clinically and hematologically and had had previous complete gastric analyses. In Table I the clinical and hematological findings in the 22 cases of pernicious anemia are recorded. The results of the gastric analyses in 16 of the 22 patients have been reported previously (1).

TABLE I

The clinical and hematological status of the 22 cases of pernicious anemia

Case number	Age	Red blood cells	Hemoglobin (Newcomer)	Daily maintenance dose of liver extract no. 343—derived from grams liver	Central nervous system involvement
	years	millions per c mm	per cent		
1	78	2.90	65		Moderate
2	62	4.89	78	Intravenous*	Advanced
3	59	4.86	84	300	Advanced
4	58	5.19	92	300	Advanced
5	54	4.54	84	Intravenous	Early
6	46	2.99	49		Early
7	55	5.64	94	300	Advanced
8	71	4.14	86	50	Early
9	49	4.93	73		Advanced†
10	58	5.10	78	Intravenous	Early
11	46	4.63	69	Intramuscular	Moderate†
12	72	5.60	86	Intravenous	Advanced
13	64	4.68	83	Intramuscular	Advanced†
14	66	5.31	110	300	Advanced
15	42	5.65	101	300	Early
16	54	5.28	83	Intravenous	None
17	48	4.73	88		Early
18	58	5.08	89	400	Moderate
19	54	1.15	25		Advanced
20	48	4.95	84	400	Early
21	62	1.73	31		Advanced
22	58	1.16	27		Advanced

* Those patients now receiving intravenous or intramuscular liver extract at weekly intervals had previously been unable to maintain the blood at normal levels while receiving at least the amount of liver extract derived from 300 grams of whole liver daily by mouth.

† Patients showing recent improvement in neurological conditions.

No food or drink was given to the subjects between the evening meal and the morning of the test. Early in the morning the fasting gastric contents were removed by means of a Rehfuß tube while the subject was in a semi-recumbent position. The subject then swallowed the tube to approximately the 75 cm. mark and was turned onto the right side. After the subject had rested in this position for from $\frac{1}{2}$ to $\frac{3}{4}$ of an hour, an attempt was made to localize the position of the tube. To localize the tube the following procedures were followed:

1. Auscultation was performed over the epigastrium during the injection of air into the tube. (As stated by Richards (22), if the tip of the tube was in the stomach a loud cavernous sound was transmitted to the ear of the examiner but if it was in the duodenum, a more distant, muffled, high pitched sound was heard.)

2 Sensation of the subject on the injection of the air was noted (Many of the patients noticed a distinct difference in sensation when the air was injected into the duodenum The injected air was felt much deeper and very quickly could be felt passing through the intestines)

3 The rapid disappearance of the acid injected was observed

4 Persistence of deep bile color to the fluid on removal was noted

If the above requirements were fulfilled, it was thought that the tip of the tube was in the duodenum and that the fluoroscope was not absolutely necessary to verify the position of the tube Fluoroscopic examination probably would have helped in several instances in passing the tube into the duodenum, as, occasionally, it was necessary to wait three hours or longer and to change the position of the subject and the tube many times before the tube dropped into the duodenum On a few occasions the examination was unsuccessful as the tube would not pass into the duodenum

After localizing the tube, 30 cc of 0.2 per cent hydrochloric acid was injected into the tube One minute later an attempt was made to remove the acid If most of the acid had disappeared at that time, continuous suction was applied to the tube after five minutes Two 30 minute samples of duodenal secretions were collected

The duodenal or gastric juice was measured in a graduated cylinder and filtered through paper The color was noted and the pH was determined colorimetrically Only the results of those samples which were neutral or alkaline were considered to be of value Silverman and Denis (15) and Wadsworth and Aaron (23) showed the value of this precaution Our own experience has also taught us that acid duodenal contents gave much lower enzyme values

Amylase and lipase were determined by the methods described by McClure, Wetmore, and Reynolds (24) However, in the lipase determination, 1 cc of duodenal juice was diluted to 5 cc with 0.33 molar phosphate buffer instead of to 50 cc Our lower lipase values may be due to the difference in the stimulus used or in the cottonseed oil emulsion

To determine the tryptic activity of the duodenal juice, 1 cc of the juice was diluted to 10 cc with distilled water, and 5 cc was used for analyses by the method of Koch and Helmer (25) described below The total tryptic content (trypsin plus trypsinogen) was determined by adding 1 cc of a 2 per cent solution of enterokinase to 1 cc of duodenal juice, making the total volume to 10 cc with distilled water, and incubating for 30 minutes at 40° C Five cc of the activated solution was then used for the analysis of its tryptic power The enterokinase was prepared from duodenal mucous membrane of hogs The mucous membrane previously had been dehydrated and defatted by means of acetone and ether

The details of the method for the determination of trypsin are as follows The casein solution, which was used as a substrate, was prepared by shaking 75 grams of Merck's casein (according to Hammarsten) with 500 cc of distilled water in a 2 liter flask until the casein was in a finely divided state Then 500 cc of 0.8 per cent sodium carbonate was added and the mixture shaken until the casein was dissolved If preserved with toluene, the solution will keep several days in the ice box

For the determination, 80 cc of the casein solution was measured into an Erlenmeyer flask of 125 cc capacity, and enough 0.4 per cent sodium carbonate was added so that the total volume was 100 cc upon the addition of the substance to be tested The casein solution was then allowed to come to 40° C and the trypsin was added The mixture was stirred well to insure homogeneity, and 25 cc was pipetted into a small beaker or flask containing 3.6 cc of

normal acetic acid and about 1 gram of talc (It is important to stir vigorously while adding the casein to the acid to insure a good precipitation) The remaining solution was allowed to digest for 4 hours at 40° C The 25 cc. of the solution above served as a blank The precipitated casein was removed by filtration through a good quantitative paper and the refractive index was read at 25° C by means of a Bausch and Lomb immersion refractometer

At the end of the digestion period, exactly 4 hours, the flask was removed from the incubator and, again, 25 cc of the solution was pipetted off and the undigested casein precipitated as before The difference in the refractive index of the blank and the 4 hour filtrate was the index of the amount of digestion The refractive index changes were recorded in scale readings of the refractometer Since the filtrate from the casein precipitation was water clear, no difficulty was encountered in securing constant readings with the refractometer

In order to have a standard with which to compare the tryptic activity a standard curve was made by determining the change in refractive index caused by quantities of U S P pancreatin, ranging from 1 mgm to 20 mgm per 100 cc of casein solution The refractive index changes caused by known concentrations of a 0.4 per cent sodium carbonate solution of U S P pancreatin are shown in Table II, and the data from Table II are plotted in Chart 1

TABLE II

Change in refractive index readings at 25° C produced by known concentrations of U S P pancreatin

Pancreatin mgm	Change in refractive index scale readings immersion refractometer
1	0.72
2	1.20
4	2.10
6	3.35
8	4.18
10	5.36
15	7.45
20	9.17

To rule out the possibility of pepsin interfering with the trypsin determination, 5 cc of normal gastric juice with a high pepsin content was allowed to act on casein in the manner outlined above The gastric juice had no proteolytic activity under these conditions

RESULTS IN NORMAL SUBJECTS

In Table III are tabulated the results in normal subjects The fasting gastric contents all contained a trace of trypsin and lipase In the samples with acid reaction the amylase was entirely absent The one specimen which was alkaline had considerable amylolytic power, due, no doubt, to the presence of saliva This sample also had the greatest tryptic activity

In the duodenal specimens in which the reaction was neutral or alkaline, the results were quite uniform Again it was noted that the acid reaction completely inactivated the amylolytic enzyme and mar¹ and¹

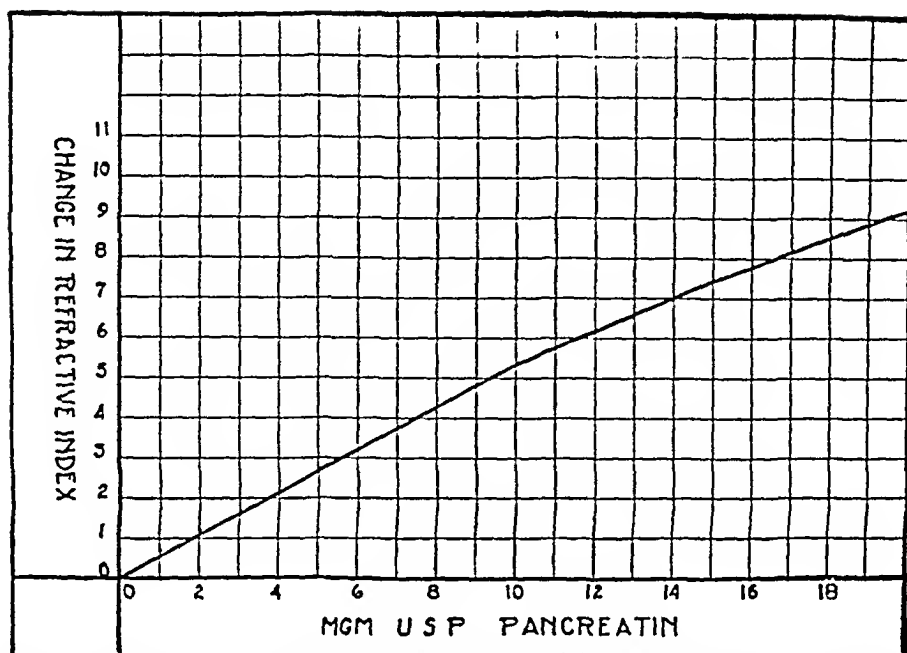


CHART 1 CHANGE IN REFRACTIVE INDEX READINGS AT 25° C PRODUCED BY KNOWN CONCENTRATIONS OF U S P PANCREATIN

TABLE III

The volume, color, pH, trypsin before and after actuation with enterokinase, lipase, and amylase in the fasting gastric juice and in the duodenal contents following acid stimulation in normal individuals

Case number	Date	Specimen	Volume	Color	pH	Tryp- sin	Tryp- sin and try p sin- ogen	Lip ase	Amyl ase
	1932		cc			mgm per cc	mgm per cc	cc N/10 NaOH	mgm glu cose
1	September 6	Fasting gastric	28	Negative	1.8	0.3	1.8	0.0	0.0
		1st half hour	80	Yellow	7.4	13.3	27.5	3.2	2.1
		2d half hour	30	Yellow	7.4	17.0	32.9	3.5	2.9
2	September 8	Fasting gastric	23	Bile	7.6	2.0	3.9	0.2	1.3
		1st half hour	95	Yellow	7.8	14.2	27.7	2.7	1.0
		2d half hour	12	Yellow	7.8	23.8	42.7	4.2	2.6
3	September 22	Fasting gastric	35	Bile	1.3	1.0	1.0	0.2	0.0
		1st half hour	64	Yellow	7.6	16.3	20.6	2.6	1.6
		2d half hour	46	Yellow	4.7	6.5	13.0	1.6	0.0
4	October 6	Fasting gastric	17	Negative	2.2	1.8	2.2	0.2	0.0
		1st half hour	110	Amber	8.0	13.2	25.2	2.8	0.9
		2d half hour	65	Amber	8.3	15.9	31.0	4.3	1.5
5	October 11	Fasting gastric	70	Bile	2.0	1.0	1.2	0.1	0.9
		1st half hour							
		2d half hour	107	Yellow	7.0	12.2	22.0	2.8	0.9

creased the lipolytic and tryptic activity. It was interesting to observe the relatively large amount of trypsinogen that was present in the normal duodenal contents. After activation, the tryptic power of the juice was increased 83 per cent. We believe that the determination of the tryptic power after activation with enterokinase may offer a more accurate index of pancreatic function, since at least two factors must be considered as playing a role in determining the amount of tryptic activity in the duodenal juice—namely, the ability of the pancreas to secrete trypsinogen and the ability of duodenal mucosa to supply enterokinase to activate it.

RESULTS IN PERNICIOUS ANEMIA

In Table IV the results of the analyses of the gastric and duodenal contents in the 22 cases of pernicious anemia are recorded. The fasting gastric samples contained only small amounts of trypsin and lipase, except in the samples containing bile where there was a noticeable increase in the amount of these enzymes in most cases. Many of the fasting gastric samples contained a greater amount of amylase than the duodenal samples. This was undoubtedly due to the presence of saliva in the fasting gastric contents, since patients with pernicious anemia have no acid in the fasting gastric contents to destroy the amylase of the saliva.

There was an increase in tryptic and lipolytic activity over the fasting gastric findings in all of the duodenal samples. The average of the tryptic activity in the half hour duodenal samples of the 22 cases of pernicious anemia, before activation with enterokinase, was 15.2 mgm per cc and 21.5 mgm per cc following the activation. The value obtained before activation was approximately the same as that found in normal persons, but the value after activation was distinctly lower. There was an increase of only 49 per cent of the tryptic value after activation with the enterokinase, as compared with the increase of 83 per cent found in the normal specimens. Therefore, it appears that there is a decreased secretion of trypsinogen in these patients, although their values for tryptic activity by the usual tests fall within the normal range. It is evident from these observations that there is a normal amount of enterokinase secreted by the duodenum. The average of the lipolytic activity in the half hour samples of the cases of pernicious anemia was slightly lower than the normal values. The average amylolytic values were approximately equal to those found in the normal persons.

The 22 cases of pernicious anemia were grouped in Table V and in Chart 2 according to their clinical and hematological status at the time of the analyses. Those patients having moderate to advanced central nervous system involvement usually had a much lower tryptic activity in the duodenal contents, not only when the tryptic values were compared with the normal findings, but even when these values were compared with those obtained in the patients with pernicious anemia having early or no

TABLE IV

The volume, color, pH, trypsin before and after activation with enterokinase, lipase, and amylase in the fasting gastric juice and in the duodenal contents following acid stimulation in 22 cases of pernicious anemia

Case number	Date	Specimen	Volume	Color	pH	Trypsin	Trypsin and trypsinogen	Lipase	Amylase
			cc			mgm per cc	mgm per cc	cc N/10 NaOH	mgm glucose
1	1932 July 6	Fasting gastric 1st half hour 2d half hour	45 17	Green Yellow		12.4	16.4	1.0	0.9 0.7
2	July 11	Fasting gastric 1st half hour 2d half hour	3 56 13	Negative	8.4 6.8 8.4	1.9 3.3 8.0	1.7 4.6 11.0	0.2 0.2 1.1	0.8 0.3 0.9
	October 12	Fasting gastric 1st half hour	13 52	Bile Amber	8.2 8.4	0.2 8.2	1.0 10.8	0.0 0.8	1.1 0.9
3	July 15	Fasting gastric 1st half hour 2d half hour	40 56 53	Negative		3.7 9.3 16.1	4.2 11.5 25.3	0.2 0.5 1.2	1.7 1.4 1.8
4	July 22	Fasting gastric 1st half hour	13 36	Negative Yellow		0.2 9.9	0.2 17.2	0.2 2.2	0.6 0.7
5	July 27	Fasting gastric 1st half hour 2d half hour	18 150 63	Negative Green Green		0.0 20.0 19.5	0.3 29.1 34.8	0.1 1.0 1.0	2.3 0.8 1.5
6	August 11	Fasting gastric 1st half hour 2d half hour	45 79 50	Yellow Yellow		2.0 17.2	2.6 24.6 30.8		0.8 0.8 1.1
7	August 30	Fasting gastric 1st half hour 2d half hour	3 120 58	Negative Yellow Yellow		0.5 9.2 12.3	0.5 15.2 16.2	0.1 0.7 1.2	0.9 2.2 2.5
8	September 1	Fasting gastric 1st half hour 2d half hour	24 75 8	Negative Yellow Yellow	8.7 7.6 7.6	0.0 29.7 24.8	0.0 35.2 38.2	0.0 3.1 3.2	1.3 0.9 3.0
9	September 7	Fasting gastric 1st half hour 2d half hour	13 44 50	Negative Brown Brown	8.4 7.6 7.8	0.6 13.5 14.2	0.7 20.0 28.3	0.0 3.2 4.4	0.9 0.8 2.1
10	September 9	Fasting gastric 1st half hour 2d half hour	88 10	Amber Brown	8.0 8.6	15.2 16.3	26.6 21.2	1.7 1.7	2.5 3.0

TABLE IV—Continued

Case number	Date	Specimen	Volume	Color	pH	Tryp sin	Tryp- sin and tryp- sino- gen	Lip ase	Amyl ase
	1932		cc			mgm per cc	mgm per cc	cc N/10 NaOH	mgm glu cose
11	September 13	Fasting gastric	16	Bile	7.8	2.7	5.3	0.9	2.9
		1st half hour	110	Amber	7.8	12.4	26.2	4.2	3.4
		2d half hour	45	Yellow	7.8	12.4	21.4	4.7	4.8
12	September 14	Fasting gastric	10	Negative	8.2	0.2	1.0	0.1	2.2
		1st half hour	95	Brown	7.8	18.8	27.2	3.4	1.9
		2d half hour	25	Brown	8.2	16.7	24.4	3.0	2.3
13	September 16	Fasting gastric	2			0.0			
		1st half hour	32	Yellow	7.8	20.8	28.0	4.0	1.1
14	September 19	Fasting gastric	41	Negative	8.2	0.4	0.5	0.2	2.5
		1st half hour	63	Brown	8.2	15.4	17.4	2.4	2.1
		2d half hour	50	Yellow	7.8	10.4	14.8	2.6	2.3
15	September 20	Fasting gastric	32	Negative	8.6	0.4	1.0	0.0	2.3
		1st half hour	30	Brown	7.8	17.2	26.0	3.4	0.8
		2d half hour	19	Yellow	8.2	15.4	22.5	3.0	1.8
16	September 21	Fasting gastric	58	Bile		2.6	5.5	0.8	3.1
		1st half hour	31	Yellow		9.5	18.2	2.2	1.5
		2d half hour	21	Blood		23.6	29.2	3.2	3.8
17	September 26	Fasting gastric	14	Bile	7.8	12.8	12.2	0.6	5.2
		1st half hour	80	Amber	7.6	38.2	34.8	3.0	2.9
		2d half hour	46	Amber	8.2	20.0	25.0	1.7	3.7
18	September 28	Fasting gastric	5	Bile	8.0	1.5			
		1st half hour	65	Amber	7.8	10.2	16.9	1.0	2.7
		2d half hour	11	Blood	8.2	8.9	13.2	1.3	2.6
19	September 29	Fasting gastric	9	Negative	8.6	0.6	1.0	0.2	3.4
		1st half hour	65	Amber	8.2	10.0	15.6	1.2	1.9
20	October 3	Fasting gastric	29	Negative	7.8	2.7	6.0	0.3	2.3
		1st half hour	129	Amber	8.0	17.4	26.6	3.0	2.3
		2d half hour	46	Amber	8.2	17.2	22.0	2.0	3.7
21	October 5	Fasting gastric	3			0.4			
		1st half hour	82	Amber	8.5	9.2	12.0	1.2	0.2
		2d half hour	50	Amber	8.4	12.8	16.0	1.2	0.2
22	October 17	Fasting gastric							
		1st half hour	22	Amber	8.6	12.8	16.9	1.8	0.9
		2d half hour	6	Amber	8.4	13.8	20.4	2.6	1.3

TABLE V

Mean values with probable error of the mean of the trypsin, trypsin plus trypsinogen, lipase, and amylase of 141 of our samples in the various groups of patients with pernicious anemia and in normal subjects

	Number of cases	Mean values			
		Trypsin	Trypsin and trypsinogen	Lipase	Amylase
I Normals	5	$\frac{\text{mgm}}{\text{per cc}}$ 15.4 \pm 0.67*	$\frac{\text{mgm}}{\text{per cc}}$ 27.2 \pm 1.62	$\frac{\text{cc}}{\text{N/10 NaOH}}$ 3.2 \pm 0.12	$\frac{\text{mgm}}{\text{glucose}}$ 1.5 \pm 0.14
II Pernicious anemia					
1 Cases having moderate to advanced central nervous system involvement	14	12.2 \pm 0.63	18.0 \pm 0.94	2.1 \pm 0.23	1.6 \pm 0.18
2 Cases having early or no demonstrable central nervous system involvement	8	20.1 \pm 1.18	27.8 \pm 1.08	2.4 \pm 0.17	2.2 \pm 0.18
3 All cases having RBC below 3.0 million per c mm	5	12.6 \pm 0.75	17.8 \pm 1.03	1.4 \pm 0.14	1.1 \pm 0.17
4 All cases having normal RBC	17	15.7 \pm 0.95	21.8 \pm 1.13	2.4 \pm 0.19	2.0 \pm 0.16
5 Cases having moderate to advanced central nervous system involvement and RBC below 3.0 million per c mm	4	11.8 \pm 0.93	16.2 \pm 0.56	1.4 \pm 0.16	1.0 \pm 0.21
6 Cases having moderate to advanced central nervous system involvement and a normal RBC	10	12.3 \pm 0.86	18.8 \pm 1.24	2.3 \pm 0.29	1.7 \pm 0.22
7 Cases having early central nervous system involvement and RBC below 3.0 million per c mm	1	17.2	24.6		1.0
8 Cases having early or no demonstrable central nervous system involvement and normal RBC	7	20.2 \pm 1.31	27.8 \pm 1.21	2.4 \pm 0.18	2.3 \pm 0.19
9 Cases able to maintain RBC at normal levels on liver extract derived from 300 or less grams of liver	6	15.4 \pm 1.61	21.8 \pm 2.04	2.1 \pm 0.31	1.6 \pm 0.16
10 Cases requiring more than the amount of liver extract derived from 300 grams of liver	9	14.6 \pm 1.05	22.4 \pm 1.52	2.4 \pm 0.28	2.2 \pm 0.23
11 All cases	22	15.1 \pm 0.79	21.6 \pm 0.95	2.2 \pm 0.16	1.8 \pm 0.14

* Probable error of mean

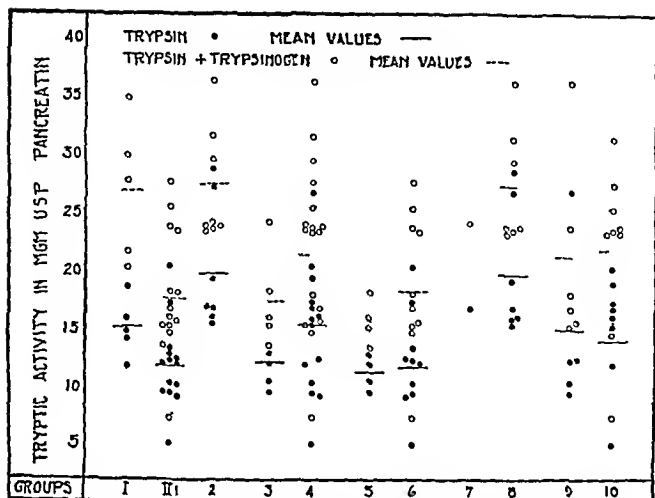


CHART 2 INDIVIDUAL VALUES OF TRYPSIN AND OF TRYPSIN AND TRYPSINOGEN PLOTTED ACCORDING TO GROUPING IN TABLE V

demonstrable central nervous system involvement. This finding was more evident when the tryptic activity of the samples after activation with enterokinase was studied. The amylolytic and lipolytic activity of the duodenal contents of the patients having moderate to advanced central nervous system involvement was also slightly decreased, but not to such a degree as the tryptic activity. One patient (Case number 2) with marked central nervous system involvement had the lowest trypsin values in the series, and, repeating the test three months later, similar results were obtained. Of the four patients having moderate to advanced central nervous system involvement, who had normal amounts of tryptic activity in the duodenal contents, three had recently shown definite improvement of symptoms referable to involvement of the central nervous system while receiving liver extract by injection.

There were only five patients having red blood counts below 3.0 million per c mm, but in all there was a definite decrease in lipase and amylase and a slight decrease in trypsin. Fifteen of the patients had been studied by this department for a sufficient length of time to be used for determining the relationship of the duodenal contents with the maintenance dose of liver extract. The averages of the tryptic and lipolytic activity in those patients who were able to maintain the blood at normal levels by taking the amount of liver extract derived from

less of whole liver daily, were approximately equal to those found in the patients requiring more liver extract. The average of the amylolytic activity of the latter group was slightly higher than that of the former. The one patient who was able to maintain the blood at normal levels while taking very small amounts of liver extract, however, had the highest tryptic activity in the duodenal contents of any of the fifteen patients.

DISCUSSION

In the 22 cases of pernicious anemia studied, there were no cases in which there was an absence of the pancreatic enzymes in the duodenal contents. This is in agreement with the findings of Crohn (7) (8), Chace and Myers (9), White (10), and McClure and associates (11), but is at variance with the findings of Cheney and Niemand (21). However, Cheney and Niemand based their opinion on the analyses of the fasting gastric contents. From our work it is evident that fasting gastric juice findings are not a satisfactory index of the external secretory function of the pancreas.

Although there were no cases in which there was a total absence of the pancreatic enzymes, it is interesting to note that, in all the patients with pernicious anemia having decreased amounts of tryptic enzymes, there was moderate to advanced central nervous system involvement present. We feel that this finding of low enzymatic activity in the duodenal contents of cases with achylia gastrica is of more significance than similar findings in cases having normal gastric function, because there was no free hydrochloric acid or pepsin to inhibit the pancreatic enzymes and, in addition, there was only a small amount of gastric juice in such cases to dilute the duodenal contents. The finding of decreased tryptic activity in the patients with pernicious anemia having moderate to advanced central nervous system involvement, suggests the possibility that a decrease in the external secretory function of the pancreas might be of etiological importance in the production of central nervous system involvement in pernicious anemia. A study of the duodenal contents of a larger series of cases is necessary to verify this possibility.

In this limited series, Case number 8 is the only one suggesting any correlation between maintenance dose of liver extract and the amount of pancreatic enzymes in the duodenal contents. This patient had one of the highest tryptic values in the whole series and was able to maintain the blood at normal levels for a period of $2\frac{1}{2}$ years while taking the amount of liver extract derived from 100 grams of whole liver every other day. This suggests that the maintenance dose of liver extract in some patients with pernicious anemia may be influenced by the degree of activity of the pancreas, however, the findings in the remaining patients show no such relationship. The probability still remains that in the majority of the patients receiving an adequate diet and having no complications, the

maintenance dose of liver extract is governed by the ability of the patient to absorb the active principle from the gastro intestinal tract, as suggested by Castle and his associates (26)

CONCLUSIONS

- 1 Twenty two cases of pernicious anemia were studied and all showed pancreatic enzymes in the duodenal contents
- 2 The determination of pancreatic enzymes in the fasting gastric contents is of no value in estimating pancreatic activity
- 3 The incubation of duodenal contents with enterokinase is necessary in order to determine the total amount of proteolytic enzymes secreted by the pancreas
- 4 The ability of the duodenal mucosa to secrete enterokinase is apparently not impaired in pernicious anemia
- 5 All patients with pernicious anemia who had decreased tryptic activity showed moderate to advanced central nervous system involvement
- 6 With the possible exception of one case, there was no correlation between maintenance dose of liver extract and the activity of the pancreatic enzymes

BIBLIOGRAPHY

- 1 Helmer, O M, Fouts, P J, and Zerfas, L G, J Clin Invest 1932 xi 1129 Gastro Intestinal Studies I Gastric Juice in Pernicious Anemia
- 2 Castle, W B, Am J M Sc, 1929, clxxviii, 748 Observations on the Etiological Relationship of Achylia Gastrica to Pernicious Anemia I The Effect of the Administration to Patients with Pernicious Anemia of the Contents of the Normal Human Stomach Recovered after the Ingestion of Beef Muscle
- Castle, W B, and Townsend, W C, Am J M Sc, 1929, clxxviii, 764 Observations on the Etiological Relationship of Achylia Gastrica to Pernicious Anemia II The Effect of the Administration to Patients with Pernicious Anemia of Beef Muscle after Incubation with Normal Human Gastric Juice
- 3 Ehrmann, R, and Lederer, R, Berl klin Wchnschr, 1908, xlv, 1450 Ueber die Wirkung der Salzsäure auf die Fermentsekretion des Magens und der Bauchspeicheldrüse
- 4 Einhorn, M, J A M A, 1910, lv, 6 Experiences with the Duodenal Contents
- 5 Einhorn, M Am J M Sc, 1914 cxlviii, 490 Direct Examination of the Duodenal Contents (also Bile) as an Aid in the Diagnosis of Gallbladder and Pancreatic Affections
- 6 Einhorn, M, Am J M Sc 1918, clvi, 817 The Fractional Examination of the Duodenal Contents
- 7 Crohn, B B, Am J M Sc, 1913 cxlv, 393 The Diagnosis of the Functional Activity of the Pancreatic Gland by Means of Ferment Analysis of the Duodenal Contents and of the Stools
- 8 Crohn, B B, Arch Int Med, 1915, xv, 581 Studies in Pancreatic Disease

- 9 Chace, A F, and Myers, V C, Arch Int Med, 1913, vii, 628 The Examination for Diagnostic Purposes of the Enzyme Activity of Duodenal Contents
- 10 White, F W, Boston Med and Surg J, 1916, clxiv, 674 Observations on the Use of the Duodenal Tube for Diagnosis and Treatment
- 11 McClure, C W, and Jones, C M, Boston Med and Surg J, 1922, clxxxii, 909 Studies in Pancreatic Function The Enzyme Concentration of Duodenal Contents in Pathological Conditions Involving the Pancreas, Liver, and Stomach
- 12 Roth, N, and Sternberg, F, Deutsche med Wchnschr, 1922, xlviii, 1207 Zur Frage der Pankreasachylie
- 13 Kahn, H, Klin Wchnschr, 1923, ii, 692 Zur Duodenalsondierung
- 14 McClure, C W, Montague, O C, and Mortimer, E, Boston Med and Surg J, 1924, cxc, 357 Pancreatic Function in the Absence of Free Hydrochloric Acid from the Stomach
- 15 Silverman, D N, and Denis, W, South M J, 1924, xvi, 549 Studies of the Pancreatic Enzymes in Fasting Duodenal Contents
- 16 Piersol, G M, and Bockus, H L, Arch Int Med, 1925, xxxv, 204 Pancreatic Enzymes in Cholecystitis
- 17 Landau, A, Cygielstreich, J, and Fejgin, M, Arch d mal de l'app digestif, 1926, xvi, 409 Achylie Gastro-Pancreatique
- 18 Landau, A, and Glass, J, Arch f Verdauungskr, 1929, xvi, 192 Achylia Gastro-pancreatica
- 19 Martin, Lay, Arch Int Med, 1927, xxxv, 343 Biliary, Pancreatic, and Duodenal Studies II Estimation of Pancreatic Enzymes and Value of Such Determinations from a Clinical Standpoint
- 20 Okada, S, Imazu, T, Kuramochi, K, Matsubara, M, Tsukahara, T, Arch Int Med, 1929, xliii, 413 Pancreatic Function III The Pancreatic Secretion in Disturbed Gastric Secretion
- 21 Cheney, G, and Niemand, F, Arch Int Med, 1932, lxi, 925 A Possible Relationship of Pancreatic Insufficiency to Addison-Biermer (Pernicious) Anemia
- 22 Richards, C V, Arch Int Med, 1929, xlv, 71 An Improved Duodenal Tube Its Advantages with a Brief Outline of Duodenal Intubation
- 23 Wadsworth, J V, and Aaron, A H, Am J Surg, 1929, vii, 480 Observations on the Enzymatic Activities of the Duodenal Contents
- 24 McClure, C W, Wetmore, A S, and Reynolds, L, Arch Int Med, 1921, xxvii, 706 New Methods for Estimating Enzymatic Activities of Duodenal Contents of Normal Man
- 25 Helmer, O M, Doctor's Thesis, University of Chicago, Sept 1927 A Study of Trypsin and its Activation by Enterokinase
- 26 Castle, W B, Heath, C W, and Strauss, M B, Am J M Sc, 1931, clxxiii, 741 Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia IV A Biologic Assay of the Gastric Secretion of Patients with Pernicious Anemia Having Free Hydrochloric Acid and That of Patients without Anemia or with Hypochromic Anemia Having no Free Hydrochloric Acid, and the Role of Intestinal Impermeability to Hematopoietic Substances in Pernicious Anemia
- Castle, W B, and Taylor, F H L, J A M A, 1931, xcvi, 1198 Intravenous Use of Extract of Liver

HEAT CRAMPS A CLINICAL AND CHEMICAL STUDY

By JOHN H. TALBOTT AND JOST MICHELSEN

(From the Fatigue Laboratory, Morgan Hall Harvard University Boston and the Six Companies Hospital Boulder City Nevada)

(Received for publication December 15, 1932)

The regulation of the interchange of water and salt in the body is a delicate and very precise mechanism. Haldane and Priestley (1) found no change in the percentage of hemoglobin in the blood after ingestion of large amounts of water plus salt. Later Priestley (2) reported a diminution in the electrical conductivity of the serum following an increased water intake and an increase of conductivity after ingestion of salt solution. Crawford (3), Adolph (4) and others confirm these findings. Because of experimental difficulties fewer data are available concerning a restricted intake of these constituents in normal men. The increased output of water and salt, however, especially in urine, sweat and vomitus, has been studied extensively. Gamble and Ross (5) found a marked reduction in body weight in dogs after experimental pyloric obstruction. This loss of weight and inability to assimilate ingesta from vomiting was essentially referable to a loss of water plus sodium and chloride ions. Sweating is another mechanism by which salt and water are lost from the body. In a temperate climate this salt loss is small, even in heavy work, and is not responsible for a fall of the salt level of the blood serum (6). In a hot climate, however, sweating may reach a magnitude sufficient to alter various constituents of the blood during a work period of several hours duration (7). If this changed composition of blood reaches sufficient magnitude, then a break down of some function of the living organism may be expected.

Clinically the failure of the human organism to cope with a high environmental temperature is generally associated with the conditions known as sun stroke, heat stroke and heat exhaustion. There is another malady less well known, but nevertheless definite in its characteristics, that is frequently seen in workers in extreme heat. This is known as heat cramps. In 1904 Edsall (8) described two cases of painful cramps admitted to the hospital and later (9) attributed them to exposure to intense heat. Miners and firemen frequently report off duty because of severe muscle cramps when working in high temperatures. This malady has been adequately described and satisfactorily named but the cause has not been clearly defined. This communication is a study of several cases of heat cramps with a simple hypothesis for their origin.

- 9 Chace, A F , and Myers, V C , Arch Int Med , 1913, *xii*, 628 The Examination for Diagnostic Purposes of the Enzyme Activity of Duodenal Contents
- 10 White, F W , Boston Med and Surg J , 1916, *clxxx*, 674 Observations on the Use of the Duodenal Tube for Diagnosis and Treatment
- 11 McClure, C W , and Jones, C M , Boston Med and Surg J , 1922, *clxxxii*, 909 Studies in Pancreatic Function The Enzyme Concentration of Duodenal Contents in Pathological Conditions Involving the Pancreas, Liver, and Stomach
- 12 Roth, N , and Sternberg, F , Deutsche med Wchnschr , 1922, *xlviii*, 1207 Zur Frage der Pankreasachylie
- 13 Kahn, H , Klin Wchnschr , 1923, *ii*, 692 Zur Duodenalsondierung
- 14 McClure, C W , Montague, O C , and Mortimer, E , Boston Med and Surg J , 1924, *cxc*, 357 Pancreatic Function in the Absence of Free Hydrochloric Acid from the Stomach
- 15 Silverman, D N , and Denis, W , South M J , 1924, *xvii*, 549 Studies of the Pancreatic Enzymes in Fasting Duodenal Contents
- 16 Piersol, G M , and Bockus, H L , Arch Int Med , 1925, *xxxv*, 204 Pancreatic Enzymes in Cholecystitis
- 17 Landau, A , Cygielstreich, J , and Fejgin, M , Arch d mal de l'app digestif , 1926, *xvi*, 409 Achylie Gastro-Pancréatique
- 18 Landau, A , and Glass, J , Arch f Verdauungskr , 1929, *xvi*, 192 Achylia Gastro-pancreatica
- 19 Martin, Lay, Arch Int Med , 1927, *xxxix*, 343 Biliary, Pancreatic, and Duodenal Studies II Estimation of Pancreatic Enzymes and Value of Such Determinations from a Clinical Standpoint
- 20 Okada, S , Imazu, T , Kuramochi, K , Matsubara, M , Tsukahara, T , Arch Int Med , 1929, *xliii*, 413 Pancreatic Function III The Pancreatic Secretion in Disturbed Gastric Secretion
- 21 Cheney, G , and Niemand, F , Arch Int Med , 1932, *xlix*, 925 A Possible Relationship of Pancreatic Insufficiency to Addison-Biermer (Pernicious) Anemia
- 22 Richards, C V , Arch Int Med , 1929, *xliv*, 71 An Improved Duodenal Tube Its Advantages with a Brief Outline of Duodenal Intubation
- 23 Wadsworth, J V , and Aaron, A H , Am J Surg , 1929, *xii*, 480 Observations on the Enzymatic Activities of the Duodenal Contents
- 24 McClure, C W , Wetmore, A S , and Reynolds, L , Arch Int Med , 1921, *xxvii*, 706 New Methods for Estimating Enzymatic Activities of Duodenal Contents of Normal Man
- 25 Helmer, O M , Doctor's Thesis, University of Chicago, Sept 1927 A Study of Trypsin and its Activation by Enterokinase
- 26 Castle, W B , Heath, C W , and Strauss, M B , Am J M Sc , 1931, *clxxxii*, 741 Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia IV A Biologic Assay of the Gastric Secretion of Patients with Pernicious Anemia Having Free Hydrochloric Acid and That of Patients without Anemia or with Hypochromic Anemia Having no Free Hydrochloric Acid, and the Role of Intestinal Impermeability to Hematopoietic Substances in Pernicious Anemia
- Castle, W B , and Taylor, F H L , J A M A , 1931, *xcvi*, 1198 Intravenous Use of Extract of Liver

HEAT CRAMPS A CLINICAL AND CHEMICAL STUDY

By JOHN H. TALBOTT AND JOST MICHELSEN

(From the Fatigue Laboratory Morgan Hall Harvard University Boston and the Six Companies Hospital Boulder City Nevada)

(Received for publication December 15, 1932)

The regulation of the interchange of water and salt in the body is a delicate and very precise mechanism. Haldane and Priestley (1) found no change in the percentage of hemoglobin in the blood after ingestion of large amounts of water plus salt. Later Priestley (2) reported a diminution in the electrical conductivity of the serum following an increased water intake and an increase of conductivity after ingestion of salt solution. Crawford (3), Adolph (4) and others confirm these findings. Because of experimental difficulties fewer data are available concerning a restricted intake of these constituents in normal men. The increased output of water and salt, however, especially in urine, sweat and vomitus, has been studied extensively. Gamble and Ross (5) found a marked reduction in body weight in dogs after experimental pyloric obstruction. This loss of weight and inability to assimilate ingesta from vomiting was essentially referable to a loss of water plus sodium and chloride ions. Sweating is another mechanism by which salt and water are lost from the body. In a temperate climate this salt loss is small, even in heavy work, and is not responsible for a fall of the salt level of the blood serum (6). In a hot climate, however, sweating may reach a magnitude sufficient to alter various constituents of the blood during a work period of several hours duration (7). If this changed composition of blood reaches sufficient magnitude, then a break down of some function of the living organism may be expected.

Clinically the failure of the human organism to cope with a high environmental temperature is generally associated with the conditions known as sun stroke, heat stroke and heat exhaustion. There is another

During the summer of 1932 members of this laboratory were investigating the physiological and pathological effects of high temperature upon the living organism. Boulder City, Nevada, as the center of operations for the construction of Hoover Dam, offered unusual opportunities for this study. This city, only seven miles from the site of Hoover Dam, is situated in the Colorado River Basin Desert. There were approximately 2500 men working on the Dam who lived in Boulder City during the recent summer¹. Figure 1 gives the daily maximum temperatures in

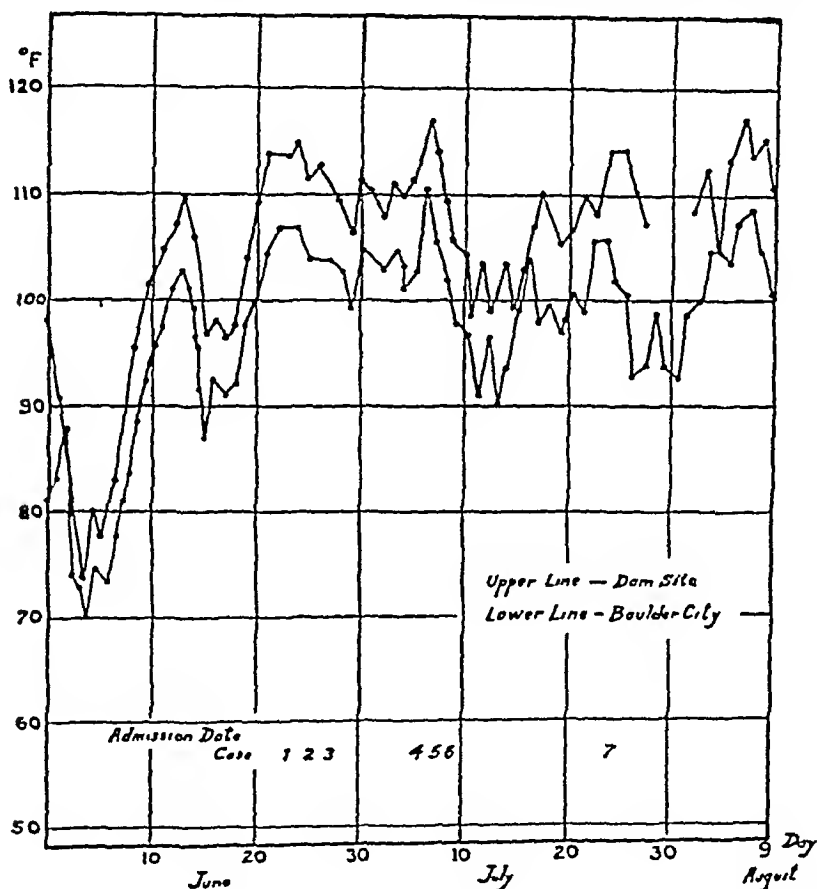


FIG 1 MAXIMUM DAILY TEMPERATURE OBSERVATIONS DURING THE SUMMER OF 1932

Boulder City and at the Dam site during this period. The admission dates for the cases discussed are noted on this figure. There were roughly four hot spells of 4 to 5 days each, and during these periods of heat all of

¹ The employees of the Six Companies Construction Company were cared for at their own Hospital. This is a modern 60-bed hospital, well equipped to care for medical and surgical cases, located in Boulder City. The authors were given a "free hand" in the diagnosis, treatment and investigation of all suspected and verified cases suffering from the ill effects of heat.

the cases were admitted to the hospital. All of the suspected heat cases were seen within 15 minutes after admission and within 45 minutes after leaving the Dam. No known medication was given before they were seen by us. Forty cc of venous blood was drawn shortly after we saw the patient, and following this a history was taken and physical examination was done. Thus the significant observations were completed before hypodermic, subcutaneous or intravenous therapy was begun.

The hospital cases admitted because of the ill effects of heat were few in number when a comparison is made with the preceding summer. While accurate statistics are not available concerning the mild and moderate cases suffering from heat, there are 17 cases in the insurance records in which death was attributed to heat from May to October, 1931. There were no deaths from heat in the summer of 1932, and according to sources considered to be reliable the number of mild and moderate heat cases was markedly below that for the summer of 1931. Greatly improved living quarters, milder temperature, abundance of cooled drinking water and acclimatization were probable factors in this reduction.

There are seven cases described in this paper. Five of these were suffering from heat cramps and two from heat exhaustion. All of the cases of heat cramps admitted to the hospital during our stay in Boulder City are included.

METHODS

The methods employed for the determination of all of the urinary constituents and the majority of the blood constituents are given in a previous paper (6). The chlorides of the serum and cells were determined according to Wilson and Ball (10). The micro method of Folin and Malmros (11) was used for blood sugar. The total fixed base of the serum was determined after Van Slyke et al. (12). The morphology of the blood was studied in the accepted clinical fashion.

Heparin was added as an anticoagulant to the drawn blood. Blood for estimation of lactic acid, sugar and formed elements was removed at once. The remainder, about 35 cc, was equilibrated at 37.5° in a water bath at the approximate value $P_{CO_2} = 40$ mm Hg. After 15 minutes equilibration, blood for measurements of oxygen capacity and CO_2 content was removed. The remaining whole blood was then centrifuged for three 20 minute periods. Calculation of cell volume at infinite time was then possible according to Hirota's method (13). The various serum determinations were performed on the separated plasma and the cell water and cell chloride on the separated cells.

RESULTS

In no instance was arterial blood secured and the equilibration of venous blood was carried out under tensions of carbon dioxide that varied somewhat in the series. Thus Table I of experimental observations is supplemented by Table II, which permits a ready and more exact comparison of the acid base equilibrium in the serum. The original data may be recalculated assuming a constant value for one of the several

HEAT CRAMPS

TABLE I
Experimental observations on oxygenated blood

Case number	Date and time	Oxygen capacity	Pco ₂	Total (CO ₂) _a	Total (CO ₂) _s	Cell volume	(Cl) _s	(Cl) _e	(H ₂ O) _e	(Total fixed base) _s	(H ₂ PO ₄) _s	(Protein) _s	(Calcium) _s	(Lactic acid) _b	(Sugar) _b	Red blood cells	White blood cells
	1923	m Eq per liter of blood	mm Hg	m Eq per liter of blood	m Eq per liter of serum	per cent	m Eq per liter of serum	m Eq per liter of cells	cc per liter of cells	m Eq per liter of serum	m Eq per liter of serum	grams per liter serum	m Eq per liter of serum	m Eq per liter of blood	mgm per 100 cc of blood	ml ions	
1	June 21 12 00 M	10.73	36.8	18.68	22.59	52.0	92.0	47.7	702	144.5	2.7	93.8	5.2	1.5	107	6.18	15,150
	June 25 1 15 P M	8.96	39.1	20.31	21.73	13.5	99.6			146.5		68.0				4.96	13,100
	June 26 7 15 A M	8.71	42.7	21.38	26.80	13.2	99.9		713	145.5	2.6	72.8	5.3	1.5	112	1.79	9,250
	July 16 5 30 A M	8.86	15.2	19.19	24.83	13.8	108.0	52.9	712	148.0	2.2	70.2		1.5	91	4.90	5,100
2	June 25 1 00 P M	8.30	36.8	19.70	21.83	41.9	109.0					68.8			116		9,300
	June 27 10 00 A M	8.10	35.7	20.52	24.42	12.6	107.8	51.6	711	151.5	2.4	67.2		1.1	109	5.12	9,250
3	July 6 11 30 A M	8.30	32.5	22.78	26.08	13.3	99.2	47.5	716	150.9	1.7	70.9	5.1	1.8	111	1.41	6,050
5	July 8 2 00 A M	9.11	15.9	19.11	25.56	19.6	99.0	51.6	722	150.5	2.4	75.6	5.1		106	4.93	7,900
	July 9 9 00 A M		35.9	19.62	23.36	49.9	101.6	53.0	728		2.2	75.2	5.2				
	July 16 1 00 P M					102.0						76.3				5.20	8,000
6	July 9 11 00 A M	8.31	37.1	16.20	18.61	45.4	96.1	54.1	738	148.3	2.5	64.0		1.5	95	4.27	12,600
	July 10 8 00 A M		35.4	16.80	19.04	43.5	100.4					72.4					
	July 11 9 00 A M	7.75	35.7	19.96	21.73	41.7	104.0	59.8	733	147.0	1.6	67.8	5.1	1.7		4.50	4,800
7	July 11 5 00 A M	7.62	12.3	19.89	29.11	40.7	105.0	57.6	723	147.2	2.2	69.5	5.1	1.5	99	1.60	10,500
	July 23 5 30 P M	9.12	42.0	18.72	23.08	47.5	94.8	50.0	721	152.2	3.5	83.2	6.5	2.9	107	5.10	
	July 21 9 00 P M	8.61							720	147.5	2.3	73.1	5.5		98	5.24	6,700

variables $pH_s = 7.45$ has been arbitrarily selected in the construction of Table II. This treatment has certain disadvantages, nevertheless its use is probably justified.

TABLE II
*Electrolytes of true plasma and r_{Cl} at $pH_s = 7.45$
(Concentrations are expressed in m Eq per liter of serum excepting r_{Cl})*

Case number	Date	(HCO_3^-)	$(Lactate^-)$	(Cl^-)	$(HPO_4^{--}) + (H_2PO_4^-)$	$(Proteinate^-)$	Σ Anions	Total base	r_{Cl}
<i>1938</i>									
1	June 24	21.12	1.5	92.7	2.69	23.1	141.1	146.1	65
	June 26	25.10	1.5	100.5	2.58	17.9	147.6	146.3	
	July 16	22.98	1.5	109.4	2.18	17.3	153.3	148.8	
3	June 27	23.13	1.1	108.2	2.44	16.5	151.4	151.8	62
4	July 6	25.27	1.8	98.7	1.72	17.5	145.0	150.6	62
5	July 8	23.52	1.5	99.0	2.39	18.6	145.0	151.5	66
	July 9	21.52	1.5	102.3	2.17	18.8	146.3		65
6	July 9	16.52	1.5	98.4	2.55	15.8	134.8	149.6	66
	July 11	22.70	1.7	105.6	1.61	16.7	148.3	148.1	71
	July 14	21.38	1.5	106.5	2.18	17.1	148.7	148.5	68
7	July 23	21.18	2.9	96.1	3.55	20.5	144.3	153.2	65

It is possible to calculate very accurately the total carbon dioxide of whole blood at $pH_s = 7.45$ with knowledge of the oxygen capacity and the partial pressure of CO_2 during equilibration (14). The spread between total CO_2 of whole blood and true plasma was assumed to be the same for a given specimen of blood at the observed pH_s and at $pH_s = 7.45$. Total CO_2 of true plasma at $pH_s = 7.45$ was calculated on this basis.

Values for pH are derived from the equation

$$pH_s = pK_s + \log (BHCO_3)_s - \log (H_2CO_3)_s,$$

where

$$pK_s = 6.10,$$

$$m \text{ Eq } (H_2CO_3)_s = 0.031 (H_2O)_s P_{CO_2},$$

and

$$(BHCO_3)_s = (\text{total } CO_2)_s - (H_2CO_3)_s.$$

Base bound by protein is calculated according to Van Slyke, Hastings, Hiller and Sendroy (15)

$$m \text{ Eq } (BP)_s = 0.104 (P)_s (pH_s - 5.08),$$

where

$$(P)_s = \text{grams of protein per liter of serum}$$

and

$$pH_s = 7.45$$

Values for serum volume and serum and cell chloride were corrected to $pH_s = 7.45$ by reference to unpublished observations on dog's blood.

The following formulae were employed

$$V_{c\text{ cor}} = V_{c\text{ obs}} + 4.0(\text{pH}_{s\text{ obs}} - 7.45),$$

$$\text{Cl}_{s\text{ cor}} = \text{Cl}_{s\text{ obs}} - 13.3(\text{pH}_{s\text{ obs}} - 7.45),$$

$$\text{Cl}_{c\text{ cor}} = \text{Cl}_{c\text{ obs}} + 20.0(\text{pH}_{s\text{ obs}} - 7.45)$$

The shift in serum volume and chloride for dog's blood within the physiological range is probably close enough to that of man to introduce no significant error in its use. Lactate of serum is calculated from its concentration in whole blood, assuming the concentration in cells and serum to be equal.

The expression \sum anions represents the corrected sum of the anions, which, if all anions were accounted for, should be approximately equal to the corresponding value for total base. Inspection of these values shows a striking difference between the blood on admission and after recovery. A marked discrepancy between total anions and cations is found only in the admission blood. Values for (HCO_3^-) , except in Case 6, are only slightly below an average normal of 24 m Eq. The lactate and inorganic phosphate of the serum are only slightly elevated, except in Case 7, where both values are about twice the normal ones. The protein ions are considerably increased because of a concentration of the blood proteins related to the degree of dehydration. Shifts of these four electrolytes of this magnitude and direction are frequently seen in a physiological state no more abnormal than that accompanying moderate exercise. It is our impression that the distribution of the chloride ion is regulated by a mechanism more resistant to change, and that an elevation or depletion of the blood chloride is seen only in disease. Thus the low values for chlorides seem to us most significant of all.

In all cases the blood taken on the second day of hospitalization after treatment had begun shows chloride values only slightly below normal. This significant rise in serum chloride coincided clinically with relief from cramps. Cases 2 and 3, who did not complain of cramps, had normal chloride values. The total base of the serum was low on admission and in Cases 1 and 6 did not reach the normal value of 152 m Eq during recovery.

The concentration of red blood corpuscles corresponds closely to the oxygen capacity and cell volume. Calculation of the cell hemoglobin gives an average value of 19.6 m Eq per liter of cells. This is slightly below the value of 20.3 given by Dill (16) for 7 normal subjects. There was some tendency for the white cells of the blood to be elevated with only slight increases in the polymorphonuclear percentage. All of the patients with elevated white counts had normal rectal temperatures.

Table III contains data concerning water balance. The fluid intake, high as it may seem, is probably considerably below the average daily intake for workmen during the hot periods. The values for salt intake

TABLE III
Daily observations on fluid intake and output

[illegible]

are only approximate but show in a striking degree the retention of salt² by the body early in recovery. This was most marked in Case 1 where the intake of 45 grams of salt in 42 hours was accompanied by a urinary excretion of only 4 grams. Only a small amount of the salt not accounted for was lost in the stools and by sweating, the remainder was retained by the body. Values for total nitrogen in the urine are high for the first two days of observation. In an unpublished study of the healthy workmen at the Dam no such increase was found in any of the 24-hour urine specimens. It seems unlikely that the high nitrogen excretion can be attributed to exercise, to the high environmental temperature or to elevated body temperature. Increased destruction of muscle protein accompanying the cramps may be the source of some of the additional nitrogen.³ The utilization of tissue protein for fuel is probably responsible for most of the increase.

CLINICAL DISCUSSION

The characteristics that distinguish heat cramps from other varieties of cramps are few. The past history may reveal a previous attack of cramps while working in a high temperature. J. C. (Case 7) was the only patient in our series that admitted having had heat cramps previously. Individual variation in susceptibility is undoubtedly important. This response is intimately related to the phenomenon of acclimatization to the heat. Adequate proof of acclimatization is not at present available but certain considerations strongly suggest this. Acclimatization seems to be complete after the 3rd to 5th day. It is our belief that any given individual is more resistant to cramps after this 5-day period, other factors being equal. Moss (17) noted that colliers acclimatized to a high temperature lost twice as much sweat under the same conditions as unacclimatized subjects. Our observations (7) do not agree with these findings.

There may be a prodromal period of 1 to 3 days before the onset of acute symptoms. During this time the salt intake may be exceeded by the salt excretion. An alcoholic bout with diminished intake of food and salt may be followed by sufficient gastric irritation and vomiting to lower appreciably the salt level of the body. In Case 1 the 3-day prodromal period was preceded by an alcoholic bout, the severity and importance of which could not be ascertained. Vomiting with loss of electrolytes preceded the muscle cramps in Cases 1, 5 and 6, and nausea was a symptom in Case 7 as well. Diarrhea may be a symptom but was not noted in

² In most instances the use of the word salt implies sodium chloride, as based on chloride determinations.

³ This process is similar to that observed by Morawitz in cases of non-nephritic uremia with hypochloremia. Morawitz, P., and Schloss, M., *Klin. Wchnschr.*, 1932, **11**, 1628. 'Extrarenale' Albuminurie und Urämie.

any of our cases of cramps. There was a prevailing impression that profuse and explosive diarrhea was an accompaniment of most of the severe heat cases in the summer of 1931. No figures are available showing the incidence of diarrhea in uncomplicated heat cramps and in other ill effects of heat. It is interesting to note that cases of cholera as described in the literature have muscle cramps associated with prolonged diarrhea. As in heat cramps the vomiting and diarrhea may seriously deplete the salt in the body.

The presenting symptom is generally pain in various groups of muscles which prevents the subject from continuing work. Tingling in the fingers and toes may precede or accompany the cramps. Edsall (9) observed extreme irritability and fibrillary twitchings of the involved muscles in his patients with heat cramps. Deep or superficial palpation produced pain. In contrast in none of our cases were there fibrillary twitchings nor did palpation produce pain. However, active motion of the affected muscles was painful in our most severe cases.

The predilection of certain groups of smooth and striated muscles for cramps is worth noting. The muscles of the calf and the flexors of the forearms were the commonest sites. The muscles of the abdominal wall were also affected in some instances quite apart from the smooth muscle of the gut. It is our belief that the vomiting and diarrhea may be referable to irritation of the smooth muscle by a process similar to that causing cramps in striated muscle. The effect of diminished blood chloride upon the heart muscle is an interesting problem for speculation. Dr. D. B. Dill suggested to us that the symptoms of Case 4 might be referred to such salt depletion. This patient came into the hospital with symptoms in some respects resembling those of coronary occlusion. His past cardiac history was not important. The physical examination was negative. His serum chlorides were below normal and he responded in a typical fashion to intravenous saline. The retention of salt during the following 24 hours is further proof of the lowered salt level of his body. The hospital does not have an electrocardiograph and no record was taken.

The relation of heat cramps to heat exhaustion or heat stroke has not been clearly defined. In common with heat stroke or exhaustion is the exposure to a high temperature and the resultant loss of large volumes of sweat. Haldane (18) found a maximum loss of $5\frac{1}{2}$ pounds per hour in seasoned colliers at work, and a much smaller loss of sweat in unacclimatized workers. That the amount of sweating is a function of external temperature rather than duration of exposure was substantiated by experiments of Hunt (19). In a Turkish bath he found that the sweat loss was about 2 pounds per hour when the temperature was between 60° and 80° C. Sweating was as free at the end of 3 hours as it was early in the experiment. This agrees with observations on ourselves during

the summer. The comparison of heat stroke with cramps and with prostration reveals less similarity beyond this point. Clinical evidence against a common causal agent in heat stroke and heat cramps is afforded by the observation made by Willcox (20). He noted that the convulsions in heat stroke were made worse after intravenous saline injections. The opposite effect might be expected if the essential pathological process were a depletion of salt and water in the central nervous system. Cramp cases show only a slight elevation of body temperature. None of the cases in our series had a temperature above 101°F after admission to the hospital. It is conceivable, but unlikely, that the temperature was higher before admission. In 37 cases reported by Gauss (21) temperatures were subnormal in most of the subjects and were not elevated in any. Cases of heat exhaustion similarly have only a slightly elevated temperature. In contrast the heat stroke or heat hyperpyrexia patients run a fever frequently as high as 110°F with a correspondingly high pulse rate (Willcox, 20). The heart rate was as intimately related to body temperature, other factors being equal, in these cases as it had previously been found to be in hot-room experiments (22). In none of our cramp cases was the pulse over 90. Restlessness and delirium were noted in one case only, Case 4, and he regained his mental equilibrium soon after admission to the hospital. In view of the number of cases diagnosed as sun stroke in the cities during the summer months, it is worth noting the absence of cases at Boulder City during the summer of 1932. Many of the men at work scaling the cliffs were exposed to the sun's rays for several hours each day. The shade temperature was frequently 45° and the temperature of the rocks was 55° to 60°C . Each day between the hours of 10 and 12 in the morning one of us (J. H. T.) regularly played three hard sets of tennis in the direct sun without any head covering. The question of whether a healthy individual, accustomed to the heat for a few days, could succumb to sun stroke is an important one and not fully answered by the cases in the literature to date.

An important criterion in substantiating a diagnosis of heat cramps is the marked relief following parenteral injection of large amounts of normal salt solution. All of our cases in this series were free from symptoms within six hours after starting treatment.

In summary, then, a diagnosis of heat cramps may be made with the following conditions satisfied: (1) Exposure to a high temperature at work. (2) Rapid loss of salt in the sweat, that is not replaced. (3) Painful muscle cramps. (4) Diminished concentration of chloride and base in the blood and likewise in the body tissues. (5) Rapid amelioration of symptoms after therapy.

PATHOGENESIS

The pathogenesis of heat cramps has been variously assigned according to the period in which the several theories were advanced. In 1904

Edsall (8) proposed, as the pathological process, acute degeneration in the muscle. Some time later an infectious agent was considered but this was never sufficiently proved. In recent years interest in this subject has been transferred to pathological physiology. In 1923 Haldane in his discussion of a paper by Moss (23) stated that he believed miners' cramps could be attributed to water poisoning. Haldane further says, "When a man is working his blood is shunted away from the kidneys and excretion of urine stops. If the kidneys were working normally they would excrete the excess of water and save the man from cramps." In consideration of our quantitative observations it is desirable to define water poisoning and to qualify the assertions concerning the excretion of urine.

The body tissues of patients with heat cramps contain hypotonic fluid as seen from Table II, but this is not from the excess of water, rather it is from the depletion of total base and chlorides. Likewise after the beginning of treatment, if salt and water are retained to make up the deficit, a gain in weight during recovery would be expected. If the fundamental process at fault were an increase of total body water, then the patients after the beginning of treatment would lose weight because of the diuretic action of salt and water. The facts observed in our cases that were weighed on admission and at discharge are as follows. Case 7 gained 3.0 pounds in the first 24 hours and Case 6 gained 7.0 pounds in 48 hours. In regard to kidney function it is our belief that the principal reason for low kidney excretion during working hours in a hot climate is peripheral dilatation of the capillaries for dissipation of heat, rather than a primary shut down of the kidneys. Heller and Smirk (24) studying rabbits and rats demonstrated a water diuresis (diuresis following an elevated fluid intake) in the presence of depleted water reserves. There is no evidence that under the above conditions for man the kidneys are not capable of excreting any excess of water that may be taken in.

Haldane (23) predicted a lowering of the blood chlorides of 3 to 4 per cent as a possible accompaniment of heat cramps. In this series the mildest case showed a 2 per cent reduction in serum chlorides and the severest case a 10 per cent reduction below the accepted normal minimal value. Direct evidence is not at hand showing that the drop in the chloride level of the blood is followed by a similar depletion of the chloride in the intercellular spaces. It is generally assumed that the concentration of electrolytes in the serum and intercellular spaces is approximately equal. If these are the facts then a loss of water and salt from the blood is followed by a partial replacement from the tissues. In the condition under discussion there is a loss of salt and water from the body with a concomitant replacement of water only. If this major process is continued, irrespective of the secondary processes, there will eventually be a lowering of the chloride level below the normal range. It is our belief that when a critical level for the chlorides is reached in working individuals

muscle cramps will occur. It seems likely that the critical chloride level is a function of individual susceptibility, acclimatization and the length of the prodromal period.

TREATMENT AND PREVENTION

If the cause of heat cramps is essentially a loss of salt and water from the body tissues then treatment should provide for restoration. The most rapid replacement of salt and water is by means of intravenous saline solution. In our study only sodium chloride was used. A larger number of cases would have enabled us to use other salts in treatment. A control study with glucose infusions without salt would likewise have been desirable. In addition to the saline infusions an exclusive milk diet was given during the first 24 hours. This simple regime was highly satisfactory in all of the cases.

It is possible to prevent cramps by providing a daily supply of salt greater than that lost in the sweat. This amount may be determined by knowing the approximate amount of chloride excreted in a 24-hour urine specimen. In our experience less than 3 grams of salt per day in the urine does not provide for a satisfactory margin of safety. A high salt diet may be provided in a number of ways. Fresh cow's milk has an average salt concentration of about 0.3 per cent and this provides fluid as well as salt. The workers of South Metropolitan Gas Co., Great Britain (25), are given a saline drink of 0.012 per cent KCl and 0.018 per cent NaCl. The number of heat cramp cases has been reduced since this regulation became effective. Salted drinking water (sodium chloride 0.25 to 1.0 per cent) has been used successfully in prevention of heat cramps among soldiers in the U. S. Army (26). Barley water and salted beer are used by certain local groups of colliers in England. It is possible that no salt need be taken between meals if the food is liberally salted at meal times. The necessary amount of salt to prevent cramps is a function of the individual's susceptibility and of the amount lost in the sweat at a given temperature.

The authors are deeply indebted to Dr. Wales Haas, Surgeon-in-Chief of the Six Companies Hospital, whose interest and cooperation made this work possible.

SUMMARY

This paper is a clinical description of five cases of heat cramps with a chemical study of the blood and urine. The hypothesis is advanced that the etiologic factor is a loss of base, chlorides and water from the body principally by way of the sweat glands without adequate replacement of the same. It is obvious that heat cramps is essentially an occupational disease and an industrial hazard is created by the association of hard work, high external temperatures and profuse sweating.

PROTOCOLS

Case 1

Mr J F, a married white man of 42, entered the hospital on June 24, 1932, complaining of vomiting for 3 days and generalized muscle cramps for 12 hours

Past history Born in Nova Scotia, he has lived for the past 28 years in southwestern United States. He has never been in the tropics, has never noted any ill effects of heat, and prefers summer to winter. He perspires freely in a warm or hot environment and his skin tans easily. He came to Boulder City June 6, 1931, and has worked since that time in the Machine Shop situated on the edge of the city. He drank at least two quarts of milk daily during the first four months of his employment in the summer of 1931. In the fall his family moved to Las Vegas and he had virtually no milk after that time. He is a light meat eater, preferring fruits and vegetables and not especially addicted to the use of salt.

Present illness Three days ago he began to vomit and, while the vomiting has diminished in severity, only a small amount of food and fluid has been tolerated during this period. Twelve hours ago cramps in the muscles of his legs first appeared and since then they have caused pain in his arms, hands, and abdominal wall. Change of position will generally bring on cramps in the parts moved, although deep pressure or palpation is not painful. He voided small amounts of urine twice during the twenty four hours before admission. He has continued to perspire freely in spite of the low fluid intake.

Physical examination Temperature, 99.6° F pulse, 88, respiration, 16 blood pressure, 130/84. The examination was essentially negative. The eye grounds were normal. Chvostek's and Trousseau's signs were negative. No fibrillary twitchings were seen.

Diagnosis Heat cramps

Treatment He was given, during the first 24 hours, two hypodermoclyses of 1500 cc of normal saline and was allowed to drink 3000 cc of milk. He received no other food or fluids.

Course The cramps ceased after 6 hours. He was sent home on the 3d day on a diet containing a minimum of one quart of milk daily. He resumed work the third day after discharge. Frequent follow ups revealed no muscle soreness or cramps during the next two periods of hot weather.

Case 2

Mr W E T, a single white male of 39, entered the hospital June 25, 1932 complaining of right sided headache and weakness in the legs of a few minutes duration.

Past history He had served in the Navy during the World War and during the past year had several radical operations on his sinuses in the Veterans Hospital in Los Angeles. He had been discharged from the hospital only one month when he was given a job on Hoover Dam. He had never lived or worked in a hot climate previously and had worked only two hours the first day of employment when he stopped because of the above complaints. His daily intake of milk was over one quart.

Present illness He had been working only two hours when he was forced to stop because of a severe right sided headache and a weakness of his legs. He did not have any muscle cramps.

Physical examination Temperature, 98.4° F pulse, 88. The scars from the previous sinus operations were the only findings of note. The reflexes were not altered.

Diagnosis Mild heat prostration

Treatment He was given, during the 24 hours in the hospital, one hypodermoclysis of 1000 cc and was allowed to drink milk ad libitum

Course He had no complaints after going to bed. His course through the summer was not followed as he did not return to work.

Case 3

Mr H. P., a single white man of 42, entered the hospital June 27, 1932, complaining of vomiting and weakness for 12 hours.

Past history He had lived from 1908 to 1910 in the Philippine Islands as a soldier in the United States Army. He had muscle cramps many times when working in the heat. The cramps were transitory and persisted no more than a few minutes with no therapy other than that of stopping work. He did not have any symptoms of heat stroke or heat exhaustion. From 1922 to 1928 he worked in a steel rolling mill in the United States and would have mild cramps at frequent intervals. He came to Boulder City in March, 1932, and has had mild muscle cramps three times since. These were in the calves of the legs or insteps of the feet. He has had no cramps for 3 weeks. His diet includes two glasses of milk daily. A warm climate is preferred to a cold one, his skin burns or tans depending upon the gradation of exposure to the sun and he perspires freely.

Present illness The patient was aware of the relatively high atmospheric temperature on the day preceding admission. He perspired freely and was aware of an increased water intake at work on this day. During the night he vomited several times and felt weak. He did not have muscle cramps.

Physical examination Temperature, 98° F., pulse, 88. Slight tenderness in the epigastrium was the most important physical finding.

Diagnosis Mild heat prostration

Treatment He was given, during the first 24 hours, two intravenous infusions of 1000 cc of normal saline and was allowed a diet exclusively of milk.

Course He did not vomit after admission to the hospital and was discharged the following day. A follow-up report 6 weeks later showed no symptoms since leaving the hospital.

Case 4

Mr W. R., a divorced white man of 42, entered the hospital on July 6, 1932, complaining of gas on the stomach and precordial pain radiating to the interscapular region.

Past history Born in Kentucky, he has lived in the west for the past 10 years. For 22 years he has been employed as a miner at frequent intervals. In 1919 and 1920 he worked in the Texas oilfields in very hot weather without any ill effects directly attributed to the heat. Fifteen years ago he had a dizzy spell accompanied by slight cardiac pain. This was very mild and he has had no cardiac complaints at any time since. He came to Boulder City in March, 1932, and since that time has worked in the tunnels.

Present illness The past week-end he went on a mild alcoholic bout and returned to work the day of admission. Soon after starting work he had gas on his stomach followed by a short period of delirium. On partially recovering from the delirium he complained of mild precordial pain radiating to the interscapular region and fear of impending death. When he was brought to the hospital he was mildly delirious.

Physical examination Temperature 99.8° F., pulse, 98, respiration, 24

blood pressure 132/84 There was mild dyspnea, precordial hyperesthesia, and the heart sounds were weak but no murmur or rub was heard

Diagnosis Heat cramps of myocardium?

Treatment He was given, during the first 24 hours an intravenous infusion of 1000 cc of normal saline in 5 per cent glucose and was allowed to drink 2000 cc of milk He received no other food or fluids

Course The patient was discharged after 24 hours and left Boulder City There is no follow up report available

Case 5

Mr R F, a single white man of 33, entered the hospital on July 8, 1932, complaining of severe pain in the calves of his legs for 2 hours

Past history Born in Tennessee he has lived the past 3½ years in the southwest He has never complained of any ill effects of heat and prefers summer to winter He drinks a great deal of water and perspires freely in the heat Since coming to Boulder City 4 months ago he has worked in the tunnels He drank 2 to 3 pints of milk daily until 2 weeks ago, at which time he stopped because of dislike for it In recent years he has not added salt to his food at the table

Present illness Ten days ago he had a mild gastric upset from which he believes he has not fully recovered He drank a small amount of beer over the holiday week end The night before admission he had slight tingling in the fingers and cramps in the leg muscles About one hour after starting work on his second shift since the holidays, he became dizzy and vomited The day was very warm and the profuse sweating was followed by consumption of large amounts of water The patient complained of such severe pains in the legs that the ambulance attendant reported the case as traumatic

Physical examination Temperature 101.0° F pulse, 88 blood pressure, 128/82 A palpable spleen was the only abnormal finding The reflexes were normal and no tenderness or fibrillary twitchings of the muscles were elicited

Diagnosis Mild heat cramps

Treatment A high milk diet was the only order

Course There was no recurrence of cramps and he was discharged after 3 days He worked for 2 weeks without any symptoms, but decided he needed a rest so he went to his home in Tennessee for the remainder of the summer

Case 6

Mr G D, a married white male of 38, entered the hospital on July 9, 1932, complaining of muscle cramps and vomiting for 3 hours

Past history Born in California he has spent a long part of his life in a warm country From 1920 to 1925 he was in Panama, while in recent years he has been working in Imperial and Death Valleys California, without any ill effects of the heat He came to Boulder City only 3 days ago and has worked outside on the Arizona Spillway for 2 days

Present illness The day before admission he felt badly at work but a headache was the only specific complaint He was slightly constipated and took a laxative with a good result some hours after He obtained a good rest on the night before admission but felt badly on going to work Muscle cramps in the arms legs and abdomen forced him to stop work and after vomiting 3 times he was sent to the hospital On admission he complained of severe headache in addition to the cramps

Physical examination Temperature, 99.8° F pulse, 83 blood pressure 118/68 The physical examination was negative

Diagnosis Severe heat cramps

Treatment He was given an intravenous infusion of 1000 cc of normal saline in 5 per cent glucose and was allowed a high milk diet

Course He had no complaints after 6 hours and next morning felt well enough to go back to work. He was discharged on the third day and returned to work the fourth day after admission. For 10 days he was symptom-free on a high salt and high milk diet. On the tenth day he fell while scaling a cliff, injuring his lumbar spine, and he did not return to work the remainder of the summer.

Case 7

Mr J C, a single white male of 38, entered the hospital on July 23, 1932, complaining of cramps for 5 hours.

Past history He was born in California and has lived for many years in a warm climate. From 1912 to 1914 he was stationed as a soldier in the Philippines and the only sickness there was dysentery. In 1929 he had mild muscle cramps while working in a hot climate. There was no recurrence. He came to Boulder City the day before admission and had only worked the first shift. In the heat he perspires very freely and has a large water intake.

Present illness Three hours after commencing work he began to have muscle cramps, first in the left hand, spreading to the legs and abdominal muscles. He noted slight nausea but no vomiting or diarrhea. There was a slight frontal headache. His water intake was large in amount.

Physical examination Temperature, 98.6° F, pulse, 84, blood pressure, 122/84. There was no muscle tenderness and no twitchings were seen. The biceps reflex was bilaterally increased. The other reflexes were present and responded normally.

Diagnosis Severe heat cramps

Treatment He was given, shortly after admission, an intravenous infusion of 1000 cc of normal saline in 5 per cent glucose. His diet during the hospital stay was milk alone.

Course The cramps ceased after 4 hours, and he was discharged the next morning. Two weeks after return to work he continued to be free of cramps.

BIBLIOGRAPHY

- 1 Haldane, J S, and Priestley, J G, *J Physiol*, 1915-1916, 1, 296. The Regulation of Excretion of Water by the Kidneys. I
- 2 Priestley, J G, *J Physiol*, 1915-1916, 1, 304. The Regulation of Excretion of Water by the Kidneys. II
- 3 Crawford, J H, *Quart J Med*, 1927, xxi, 71. Observations on the Excretion of Water and Chloride after their Oral Administration.
- 4 Adolph, E F, *J Physiol*, 1921, lv, 114. The Regulation of the Water Content of the Human Organism.
- 5 Gamble, J L, and Ross, S G, *J Clin Invest*, 1924-1925, 1, 403. The Factors in the Dehydration Following Pyloric Obstruction.
- 6 Talbott, J H, Fölling, A, Henderson, L J, Dill, D B, Edwards, H T, and Berggren, R E L, *J Biol Chem*, 1928, lxxviii, 445. Studies in Muscular Activity. V. Changes and Adaptations in Running.
- 7 Dill, D B, Bock, A V, and Edwards, H T (In Preparation). Mechanisms for Dissipation of Heat in Man and Dog.
- 8 Edsall, D E, *Am J M Sc*, 1904, cxxviii, 1003. Two Cases of Violent but Transitory Myolymia and Myotonia Apparently Due to Excessive Hot Weather.

- 9 Edsall, D E, J A M A, 1908, li, 1969 A Disorder Due to Exposure to Intense Heat.
- 10 Wilson, D W, and Ball, E G, J Biol Chem 1928, lxxix, 221 A Study of the Estimation of Chloride in Blood and Serum
- 11 Folin, O and Malmros, H, J Biol Chem, 1929 lxxxiii, 115 An Improved Form of Folin's Micro Method for Blood Sugar Determinations
- 12 Van Slyke D D, Hiller, A, and Berthelsen K C, J Biol Chem, 1927, lxxiv, 659 A Gasometric Micro Method for Determination of Iodates and Sulphates and Its Application to the Estimation of Total Base in Blood Serum
- 13 Hirota, K, J Biophys (Japan), 1925, i, 233 The Determination of Cell Volume
- 14 Henderson, L J, Bock, A V, Dill, D B, and Edwards, H T, J Biol Chem, 1930, lxxxvii, 181 Blood as a Physicochemical System IX The Carbon Dioxide Dissociation Curves of Oxygenated Human Blood
- 15 Van Slyke, D D, Hastings, A B, Hiller, A, and Sendroy, J, Jr J Biol Chem, 1928, lxxix, 769 Studies of Gas and Electrolyte Equilibria in Blood XIV The Amounts of Alkali Bound by Serum Albumin and Globulin
- 16 Dill, D B, Bock, A V, van Caulaert, C, Fölling A, Hurxthal L M, and Henderson L J, J Biol Chem, 1928, lxxviii, 191 Blood as a Physicochemical System VII The Composition and Respiratory Exchanges of Human Blood during Recovery from Pernicious Anemia
- 17 Moss K N, Proc Royal Soc London, B, 1923-1924, xcv, 181 Some Effects of High Air Temperatures and Muscular Exertion upon Colliers
- 18 Haldane, J S Brit M J, 1923, i 986 Water Poisoning
- 19 Hunt, E H J Hygiene, 1912, xi, 479 The Regulation of Body Temperature in Extremes of Dry Heat
- 20 Willcox W H, Brit M J, 1920, i, 392 The Nature, Prevention and Treatment of Heat Hyperpyrexia
- 21 Gauss, H, and Meyer, K A, Am J M Sc, 1917, cliv 554 Heat Stroke Report of One Hundred and Fifty Eight Cases from Cook County Hospital, Chicago
- 22 Dill, D B Edwards, H T Bauer, P S, and Levenson, E J, Arbeitsphysiol, 1931, iv, 508 Physical Performance in Relation to External Temperature
- 23 Moss, K N Tr Inst Min Eng 1923-1924, lxxvi, 284 Some Effects of High Air Temperature upon the Miner
- 24 Heller, H and Smirk F H J Physiol, 1932, lxxvi, 1 Studies Concerning the Alimentary Absorption of Water and Tissue Hydration in Relation to Diuresis
- 25 Oswald R J W, Lancet, 1925 i, 1369 Saline Drink in Industrial Fatigue
- 26 McCord, C P, and Ferenbaugh T L Mil Surgeon 1931, lxi, 608 Fatigue in Soldiers Due to Chloride Losses Replacement through the Use of Sodium Chloride in Drinking Water

STUDIES OF GASTRIC PEPSIN I METHODS OF MEASUREMENT AND FACTORS WHICH INFLUENCE IT

BY ARNOLD E OSTERBERG FRANCES R VANZANT¹
AND WALTER C ALVAREZ

*(From the Section on Chemistry and the Division of Medicine The Mayo Clinic
Rochester, Minn)*

(Received for publication December 23 1932)

The study of gastric pepsin has failed to receive adequate consideration for several reasons, but mainly because of the lack of technic for obtaining reliable measurements. We investigated the method originally proposed by Gates (1) and more fully elaborated by Gilman and Cowgill (2), and found it to be accurate and highly satisfactory. In fact, it is more accurate than is needed in clinical practice. We have used this method, with slight modifications, in the investigations reported in this and the following paper. The details of the technic can be obtained from the original article by Gilman and Cowgill (2). In this method one measures the rate of digestion of gelatin from an exposed and developed photographic film. As digestion proceeds, the silver granules in the gelatin are liberated, and the resulting decrease in opacity of the film is taken as a measure of the digestion which has occurred.

TECHNIC

We use, for the digestion, a cell 11 mm in diameter and 12 mm in depth turned out of sheet bakelite on a lathe. Its capacity is approximately 0.5 cc. The solution to be tested, diluted with an equal volume of glycine hydrochloric acid buffer of pH 2, is placed in the cell. A small square of the prepared film is then laid over the top of the cell and this is covered by a glass square. The whole is clamped together by means of a spring clothes pin and placed in a water bath the temperature of which is maintained at 25° C. After fifteen minutes the film is removed, washed, and dried, and the change in opacity is determined with a colorimeter. The standard for comparison is a freshly prepared suspension of colloidal silver. The index of peptic digestion is obtained by the use of a nomogram as described by Gilman and Cowgill (2).

¹ Fellow Josiah Macy, Jr. Foundation, on duty at The Mayo Foundation, Rochester, Minnesota

The Unit of Measurement

We have not found it necessary to conduct the determinations in triplicate because of the close agreement of duplicate determinations. Softening of the film due simply to moistening with the solution causes an error of approximately 70 units which must be subtracted from each determination. Since films prepared at different times may differ slightly in this respect, it is well to determine the correction for each lot of film prepared.

The unit of pepsin arbitrarily established by Gilman and Cowgill represents the proteolytic activity of a 1 : 1,000,000,000 solution of a theoretically pure pepsin. They arrived at this unit by assuming that a 1 per cent solution of Armour's 1 : 10,000 pepsin powder contains 1,000 units.

Because of possible variations in the strength of different lots of the commercial pepsin used it is well always to standardize each new lot against the one previously employed. It has been our experience that a solution of commercial pepsin is not sufficiently stable to be used from day to day. The standard must therefore be prepared each time determinations are to be made. The pepsin in the gastric contents of man and dogs is much more stable, and retains its strength for at least a week if kept at ice box temperature.

Before taking up a systematic study of gastric pepsin in various diseases it was necessary first to determine the best conditions for obtaining the juice for analysis. First we studied the residuum aspirated in the morning, before taking food or drink, but we found the concentration of pepsin in such juice so variable that single determinations had little value. Actually in specimens from twenty-four normal, fasting persons it varied from 5 to 1,930 units, with an average of 388 units. This extreme variability is doubtless due to the variation in the size of the fractions of swallowed saliva, regurgitated duodenal secretion, and gastric juice which make up the material obtained. In subsequent work we used gastric contents removed one hour after the taking of eight arrowroot cookies and 400 cc of water. We examined, in this way, juice from eighty-five persons whom we believed to be normal inasmuch as they had no complaint referable to the upper part of the digestive tract, or disease which might affect gastric secretion. Considerable variation existed in the content of pepsin, the value ranging from 0 to 580 units. The modal or most typical value was about 100 units. The arithmetic mean was distinctly higher than this, due to a few high readings. For the entire group the mean was 145 units, with a standard deviation of 125 units. From Figure 1 it can be seen that the men had slightly higher values than the women. This is in accord with the higher acidity in males which has previously been demonstrated (3). Although the variability in the read-

ings obtained after an Ewald type of meal is high, it is considerably less than that found with juice of fasting subjects

An effort was made to determine the range of variation in repeated estimations in the case of one individual. Eight determinations made on successive days in a case of pseudo ulcer associated with high pepsin readings ranged from 610 to 1,930 units, giving a mean value of 1,175 and a standard deviation of 480 units. In this case seventeen estimations of pepsin in the fasting juice ranged from 410 to 2,530 units, giving a

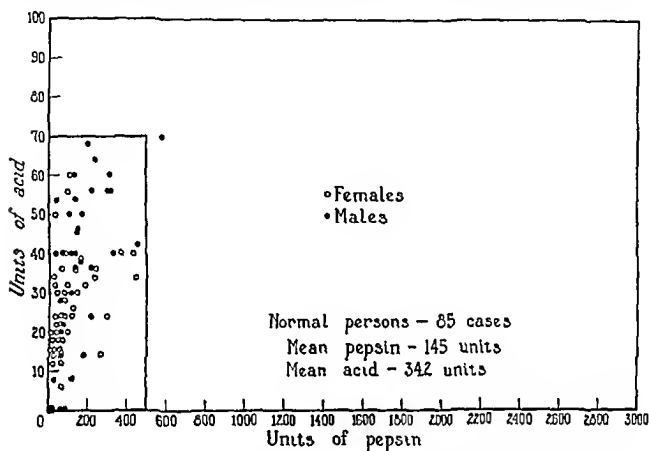


FIG 1 DISTRIBUTION OF CONCENTRATIONS OF ACID AND PEPSIN AFTER EWALD TYPE OF TEST MEAL IN EIGHTY FIVE NORMAL PERSONS

The brackets indicate normal limits for acid and pepsin

mean of 1,319, and a standard deviation of 592 units. Repeated tests on the gastric residuums from a group of twenty three patients, and a similar study of juice obtained after test meals given to three patients showed somewhat less variability. In the face of such marked variations in the values obtained from one person when examined on several days, it is obvious that before one can call any reading abnormal it must be well beyond the range of normal figures.

Effect of Contaminants

Before one can evaluate differences in concentration of pepsin it is necessary to know what effect, if any, is likely to be produced by the presence of a number of common contaminants of the gastric juice. The necessary investigations were made both *in vitro* and *in vivo*. The sub

stances which we felt might be of some importance because of their frequent occurrence in gastric contents were bile, bilirubin, mucin, blood and its several constituents such as plasma, serum, erythrocytes and crystalline hemoglobin, protein in the form of egg albumen, and peptones. As can be seen in Table 1, all of these substances caused more or less reduction in peptic activity.

TABLE 1

*The effect of various substances on the activity of pepsin solution **

After adding	Units
Dried bile (0.5 cc.)	59
Bilirubin (20 mgm.)	92
Mucin (50 mgm.)	81
Dried plasma (0.5 cc.)	150
Dried plasma (0.5 cc.)	127
Dried serum (0.5 cc.)	96
Washed erythrocytes, dried (0.1 cc.)	100
Crystalline hemoglobin (50 mgm.)	152
Egg albumen (20 mgm.)	163
Egg albumen (50 mgm.)	70
Hard boiled egg in one mass (50 mgm.)	272
Hard boiled egg finely divided (50 mgm.)	100
Witte's peptone (20 mgm.)	132
Witte's peptone (50 mgm.)	68

* In each experiment a weighed amount of the substance to be tested was added to 1 cc. of a solution of pepsin, the activity of which corresponded to 500 units. The figures represent the depressed values obtained after incubation. The amount of injury to peptic activity was represented in each case by the difference between the reading obtained and 500.

It is often stated in the literature that the presence of blood in the gastric contents will decrease proteolytic activity, and that this is due to an antipepsin. Table 2 shows that the ingestion of blood markedly lowered the proteolytic power of gastric contents, but this effect was followed by a stimulation of secretion which soon compensated for the preliminary drops. Since a similar effect was produced by raw liver, and also by beef extract, it is doubtful if the decrease of peptic activity in the presence of blood is due to a specific antipepsin. The same decrease may be found *in vitro* after the addition of crystalline hemoglobin or other fairly pure constituents of the blood (Table 1). The degree of reduction in activity seems to be roughly a function of the amount of organic material added. The possibility presents itself that the decrease may be due simply to an adsorption which results in the removal of pepsin from solution.

As is well known, the effect of raising the pH is to reduce peptic activity, and an alkaline reaction actually destroys much of the ferment. The effect of alkali when taken into the stomach of a human being is shown in Table 2, experiment 5. Following the ingestion of two Sippy

TABLE 2
Experiments in vivo on a patient with a high peptic value

Experiment 1		Units
Fasting specimen		1410
Thirty minutes after ingestion of 5 cc of dried cow s blood in capsule		620
Sixty minutes after ingestion of 5 cc of dried cow s blood in capsule		510
Ninety minutes after ingestion of 5 cc of dried cow s blood in capsule		820
One hundred twenty minutes after ingestion of 5 cc of dried cow s blood in capsule		820
Experiment 2		
Fasting specimen		1850
Thirty minutes after ingestion of 10 cc of human blood		184
Thirty minutes after ingestion of second 10 cc of human blood		910
Experiment 3		
Fasting specimen		1520
Thirty minutes after ingestion of Liebig extract		484
Sixty minutes after ingestion of Liebig extract		1320
Ninety minutes after ingestion of Liebig extract		810
One hundred twenty minutes after ingestion of Liebig extract		1520
Experiment 4		
Fasting specimen		950
Thirty minutes after ingestion of 60 grams raw liver		165
Sixty minutes after ingestion of 60 grams raw liver		420
Ninety minutes after ingestion of 60 grams raw liver		514
One hundred twenty minutes after ingestion of 60 grams raw liver		734
Experiment 5		
Fasting specimen		2040
Thirty minutes after ingestion of two Sippy tablets		180
Sixty minutes after ingestion of two Sippy tablets		1200

tablets, containing a total of 30 grains (2 grams) of calcium carbonate and 20 grains (1.30 gram) of sodium bicarbonate, peptic activity was markedly decreased, and the return to the previous reading was slow. This depressing effect of the alkali was the more striking because the experiment was performed on a subject whose normal peptic activity is unusually marked.

SUMMARY

It has been found that the Gates method (1) as modified by Gilman and Cowgill (2) gives accurate and reproducible values for the proteolytic activity of a specimen of gastric contents.

The variability in readings obtained when successive specimens of fasting residuum from the same subject were used was so great as to destroy their clinical value. More consistent results were obtained with juice obtained after an Ewald type of meal. Standards of normal for pepsin after this type of test meal are published in this paper.

Bile, mucin, blood proteins, albumin, peptones, and alkalies markedly reduced peptic activity. The fact that these substances may modify

peptic activity must be kept in mind in making a clinical study of gastric pepsin

BIBLIOGRAPHY

- 1 Gates, F L , Proc Soc Exper Biol and Med , 1927, *xxv*, 936 A Method of Proteolytic Enzyme Titration
- 2 Gilman, Alfred and Cowgill, G R , J Biol Chem , 1930, *lxviii*, 743 The Determination of Peptic Activity an Examination and Application of the Gates Method of Proteolytic Enzyme Titration
- 3 Vanzant, Frances R , Alvarez, W C , Eusterman, G B , Dunn, H L , and Berkson, Joseph, Arch Int Med , 1932, *xli*, 345 The Normal Range of Gastric Acidity from Youth to Old Age an Analysis of 3,746 Records

STUDIES OF GASTRIC PEPSIN II SECRETION OF PEPSIN IN CASES OF DUODENAL ULCER AND PSEUDO-ULCER

By FRANCES R. VANZANT¹ ARNOLD E. OSTERBERG
WALTER C. ALVAREZ AND ANDREW B. RIVERS

*(From the Section on Chemistry and the Division of Medicine, The Mayo Clinic
Rochester, Minn.)*

(Received for publication December 23 1932)

Few articles on concentration of pepsin in the gastric juice of patients with disease of the stomach and duodenum are to be found in recent literature, and the results reported by different observers are not always in accord. We shall consider here only the concentration of pepsin in the gastric juice of persons suffering with the syndrome of peptic ulcer.

Wilcox (5), in 1908, reported that the concentration of pepsin in the gastric juice of persons with peptic ulcer and hyperacidity was usually higher, and never lower, than normal. Boas (1), in 1925, and Hirsch-Mamroth and Rindfleisch (3) likewise found that the concentration of pepsin was elevated in the presence of ulcer, provided blood was not present in the sample examined, but they quoted other workers who at times failed to find any increase. Pollard and Bloomfield (4) reported that the range of concentration of pepsin was the same in patients with ulcer as in normal persons.

We have estimated the concentration of pepsin by a modification of the Gilman Cowgill method (2), described in the preceding article (6). As an arbitrary standard of measurement a 1 per cent solution of Armour's 1:10,000 commercial pepsin was assumed to contain 1,000 units.

In the greater part of the investigation we examined gastric contents removed one hour after the ingestion of a test meal which consisted of eight arrowroot cookies and 400 cc. of water. We also determined the content of pepsin in the juice removed from the stomach in the morning, before breakfast. Although the same general conclusions may be drawn from studies of contents removed after a test meal and of juice from the fasting stomach, it is necessary to consider the results obtained with the two technics separately because of differences observed in the absolute values for pepsin.

Duodenal ulcer

The diagnosis of duodenal ulcer was made in 274 cases on the basis of the history and fluoroscopic examination. In fifty-seven cases the

¹ Fellow Josiah Macy, Jr., Foundation, on duty at The Mayo Foundation, Rochester, Minnesota.

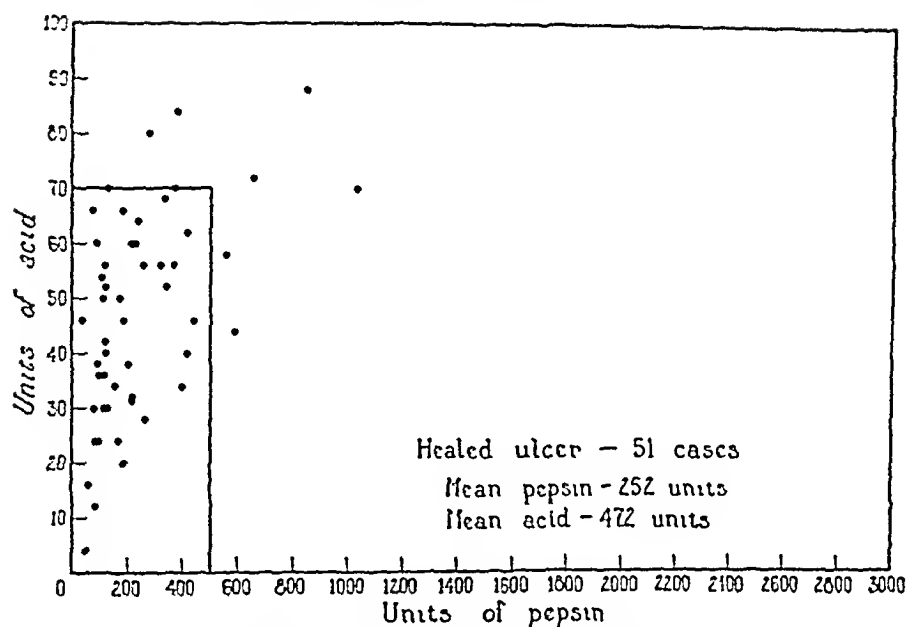


FIG 1 THE DISTRIBUTION OF ACID AND PEPSIN IN HEALED DUODENAL ULCER
The bracket indicates limits of normal

diagnosis was verified at operation. In fifty-one of the 274 cases a diagnosis of duodenal ulcer had been made, the duodenal cap was still deformed, but symptoms of ulcer had not been present for a number of years. Most of the pepsin readings in these cases of presumably healed ulcer (Fig 1) fell within normal limits, but the arithmetic mean was 252 units as compared with 145 units for the normal group (Table 1).

TABLE 1
Ewald test meal

	Number of cases	Pepsin in units		Free acid in terms of cc of normal sodium hydroxide	
		Mean	Standard deviation	Mean	Standard deviation
Normal	85	145	125	34.2	12.8
Duodenal ulcer - entire group	274	358	343	55.5	18.0
Healed	51	252	209	47.2	18.8
Mild	29	292	334	54.9	18.3
Moderate	92	332	312	55.3	16.2
Severe	28	824	505	62.2	17.2
Surgical	77	423	300	58.9	17.0
Hemorrhagic type	17	443	272	63.8	14.2
Multiple ulcers	15	398	273	61.1	15.8
Penetrating type	23	407	238	62.1	16.4
Obstructing type	11	198	116	51.1	14.2
Gastric ulcer	13	230	173	46.3	19.6
Ulcers recurring after operation	18	476	341	55.2	20.8
Pseudo-ulcer	47	467	354	49.6	18.0

In the remaining 223 cases the patients complained of active symptoms of duodenal ulcer. The mean value for pepsin presented by these patients was 419 units, which is approximately two and five-tenths times the mean value for normal persons. The readings for pepsin in many cases fell far outside the normal limits.

We next looked to see if there might be a difference between the average concentration of pepsin in cases of mild and severe ulcer. The difficulty, of course, was to find satisfactory criteria for division of the cases into groups. We finally decided to classify the cases according to the treatment which was required. We classified as mild those cases in which the symptoms were so trivial and so easily controlled that no treatment of any kind was indicated, and as moderately severe that large group in which medical treatment gave relief. The mean value for pepsin in the group with mild symptoms was 292 units, only slightly higher than that found among patients with healed ulcer, in the group of patients with more serious trouble it was 332 units (Fig 2).

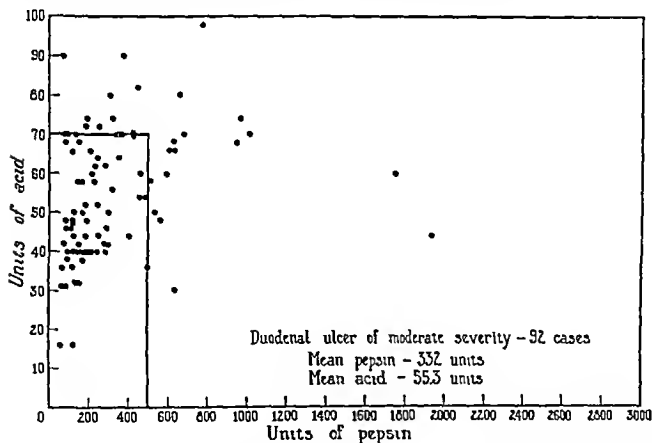


FIG 2 THE DISTRIBUTION OF ACID AND PEPSIN IN DUODENAL ULCER OF MODERATE SEVERITY
The bracket indicates limits of normal

We considered as severe those cases in which symptoms persisted in spite of the most careful treatment in the hospital. Almost all of the values for pepsin in this group fell outside the limits of normal (Fig 3), and in many instances very high readings were obtained. The mean value was 824 units, more than two and five tenths times that seen in the ordinary case of duodenal ulcer, and nearly six times that seen in normal

persons In this group we did not include cases in which there was hemorrhage or a tendency to perforation because such complications usually constituted indications for surgical treatment We did include many of those cases in which the patient was of a tense, nervous, "go-getter"

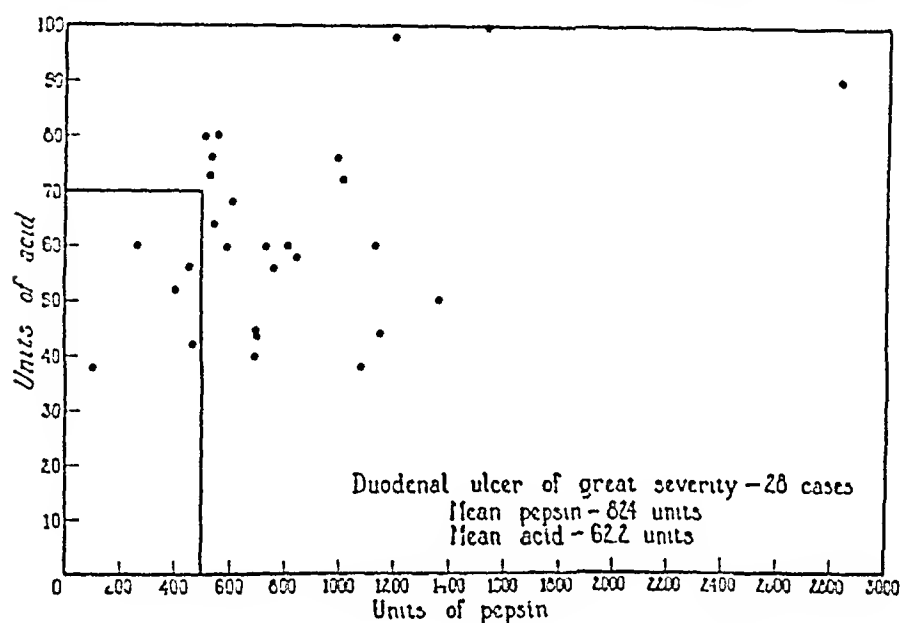


FIG 3 THE DISTRIBUTION OF ACID AND PEPSIN IN SEVERE DUODENAL ULCER
 The bracket indicates limits of normal

type, the sort of patient whom gastro-enterologists and gastric surgeons have come to recognize as a poor subject for either medical or surgical treatment It was in this type of case that the highest readings were obtained

Seventy-seven patients were advised to submit to surgical treatment either because of the presence of some complication or because of the chronicity or intractability of the disease In this group the average value for pepsin was 423 units, or about 100 units higher than the average for the medically treated patients

On analyzing the surgical cases we found a hemorrhagic type of ulcer in seventeen, multiple ulcers in fifteen, and penetrating ulcers in twenty-three cases In these three groups the values for pepsin were somewhat higher than among patients who had a less serious form of the disease The mean for bleeding ulcers was 443 units, for multiple ulcers it was 398 units, and for penetrating ulcers it was 407 units Among the group of patients treated surgically there were eleven with some cicatricial obstruction at the pylorus In most of these cases the ulcer was chronic, in some it appeared to be healed In the cases in which there was moderate

obstruction the mean value for pepsin was even lower than in the group of patients classed as having healed ulcers, namely, 198 units. It should be noted here that all cases of ulcer in which there was gross retention of gastric contents were excluded from this study. This was to avoid the complication of marked dilution of the gastric juice.

Fasting juice We estimated the concentration of pepsin in the fasting juice of forty patients with duodenal ulcer. They were divided into two groups: first, those with mild, and second, those with intractable, ulcer. It was found that the twenty-four who responded readily to treatment had an average value for pepsin of 537 units, as compared with 388 units for normal persons, those with severe symptoms had an average of 1,592 units (Table 2). It is evident that the pepsin in the fasting juice

TABLE 2
Fasting juice

Diagnosis	Male	Female	Pepsin units		Free HCl*	
			Mean	Standard deviation	Mean	Standard deviation
Normal	13	11	388	524	13	19
Moderately severe ulcer	18	6	537	617	27	24
Severe ulcer	16		1592	1020	43	23

* In terms of cc of normal sodium hydroxide per liter

with the different types of ulcer varies in the same way that it does in the juice after a test meal, except that the actual values are higher and the individual variation is greater.

Pseudo ulcer

A study was made of values for pepsin in a group of forty-seven patients who had symptoms sufficiently suggestive of ulcer to justify gastric analysis and fluoroscopic examination of the stomach. In every case the presence of atypical features in the history and the inability of the roentgenologist to detect any deformity in the stomach or cap caused the clinicians to make a diagnosis of pseudo ulcer. The spot diagram (Fig. 4) shows that the distribution of values for pepsin is similar to that in cases of ulcer and that some figures are so high as to resemble closely those found in the presence of the most intractable ulcer.

In many of the cases there was close correlation between the concentration of pepsin in the stomach and the severity of the symptoms. Just as in the case of patients with actual ulcer, there was striking correlation between the amount of pepsin and the degree of tenseness and nervous activity shown by the individual.

In six cases in which the diagnosis of pseudo-ulcer was confirmed by the inability of the surgeon at the time of operation to find an ulcer, determinations of pepsin made on fasting juice disclosed an average value of 880 units as compared to the normal mean fasting value of 388 units

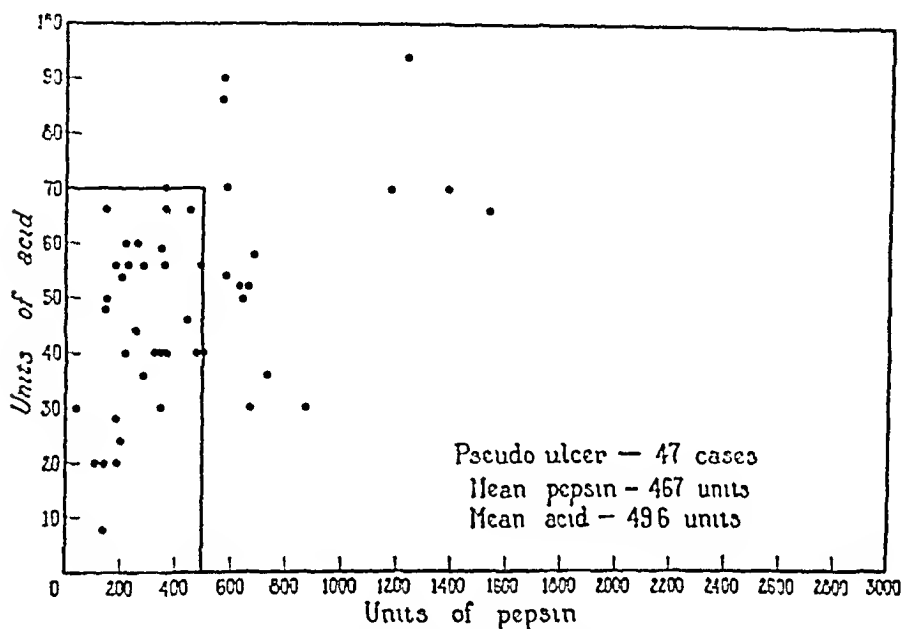


FIG 4 DISTRIBUTION OF ACID AND PEPSIN IN PSEUDO-ULCER
 The bracket indicates limits of normal

Gastric ulcer

The diagnosis of gastric ulcer was made in thirteen cases on the basis of roentgenologic examination or surgical exploration. The mean value for pepsin was 230 units, which is slightly higher than normal.

Recurrent and gastrojejunal ulcers

There were eighteen patients who, after an operation for relief of peptic ulcer, returned with a new ulcer, either in the duodenum or at the stoma made by gastro-enterostomy. The mean value for pepsin was 476 units. This figure is about one-third higher than that found by us in examination of patients with duodenal ulcer for whom operation was advised. The fact that in many of these cases the gastric juice was diluted by intestinal juice regurgitating through the gastro-enteric stoma makes it seem probable that the secretion of pepsin was even greater than the figures would indicate. It is of interest that the remaining part of the stomach of one of these patients who had undergone extensive gastric resection secreted enough pepsin to push the concentration up to 850 units.

The study of pepsin is of more clinical value than the study of acid

We were led to a study of gastric pepsin by the fact that measurement of acid has so far been of so little decisive clinical value. The question now is: Will the differences which we have found in the secretion of pepsin in different diseases be of any more value than are the measurements of acidity? Several of the early workers felt that there is such a high degree of correlation between the acid and the ferment that little advantage can be gained from determining pepsin except in those cases in which it is desirable to distinguish between achlorhydria and true achylia. Our observations lead us to disagree with this view. Although it is true that high concentrations of pepsin are commonly associated with hyperacidity, and low concentrations with hypoacidity, the rule is by no means invariable, as the coefficients in Table 3 show.

TABLE 3

Relation between concentration of pepsin and free hydrochloric acid in gastric juice

	Correlation coefficient
Fasting juice	
Normal persons	0.72 \pm 0.11
Patients with duodenal ulcer	0.85 \pm 0.12
Juice obtained after Ewald test meal	
Normal persons	0.51 \pm 0.08
Patients with duodenal ulcer	0.65 \pm 0.13
Juice obtained after stimulation by histamine	
Patients with duodenal ulcer	0.60 \pm 0.04

The fact that these coefficients are far from unity shows that often the acid titer and the amount of pepsin do not vary in the same way or to the same degree. When this happens the way is left open for the possibility that a study of the pepsin, another constituent of the gastric juice, will give information of clinical value which was not supplied by titration of the acid alone.

A glance at Figure 5 reveals that although there are definite differences between the mean acidity of normal persons and that of patients with mild and with severe ulcer, the distributions overlap so closely that in only a few cases will the physician be able to prophesy, from the gastric acidity alone, which ulcers will be hard to treat. On turning now to the curves representing values of pepsin in the three groups of cases, one sees that there is less overlapping of the distributions and when one obtains a reading higher than 800 or 900 units in a case of ulcer, one can be almost certain that the ulcer will be difficult to cure. Unfortunately it is not possible with the help of determinations of pepsin to distinguish patients with pseudo ulcer from those with real ulcer.

It should be noted, also, that whereas it is almost impossible for anyone to have a free acid titer more than twice the normal average,

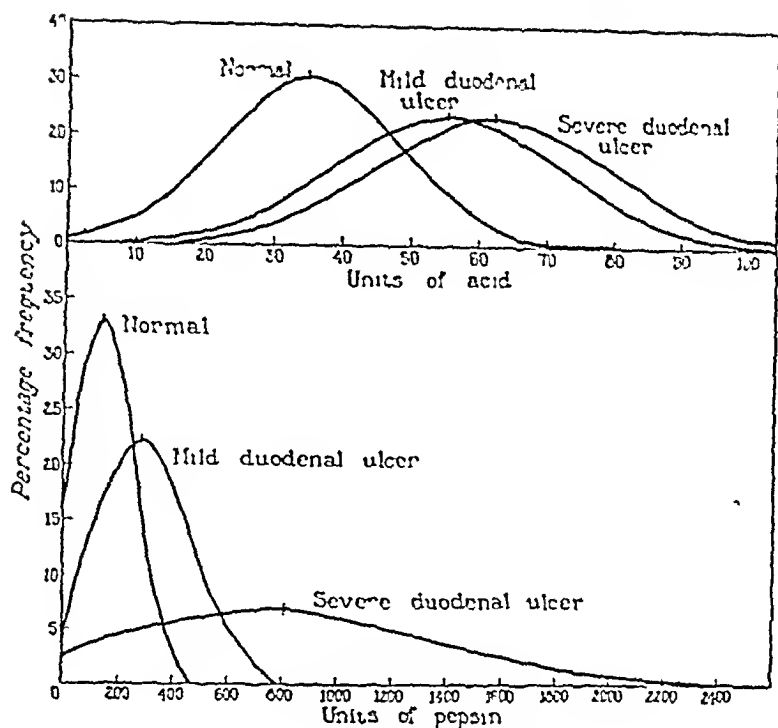


FIG 5 DISTRIBUTION OF PEPSIN AND ACID IN NORMAL PERSONS AND IN PATIENTS WITH MILD AND SEVERE ULCER

readings for pepsin occur from fifteen to twenty times the normal average. It is this widening of the distribution of the measurements made in cases of disease, a widening that carries so many of the figures beyond the possible range of normal, which makes us hopeful that the test will have decided value in clinical practice.

SUMMARY

Values for pepsin are usually increased in the presence of peptic ulcer, and there is a high degree of correlation between the amount of the ferment and the severity and intractability of the symptoms. Unfortunately the determination cannot be used to prove the presence of ulcer because very high values for pepsin are commonly found in cases in which patients are tense and nervous, and present a syndrome resembling that of ulcer. Its main value, therefore, seems to be in estimating the prognosis in cases of known ulcer.

BIBLIOGRAPHY

1. Boas, I, *Deutsche med. Wchnschr.*, 1925, I, 511. Eine neue Methode der Pepsinbestimmung im Mageninhalt.
2. Gilman, Alfred and Corrigill, G. R., *J. Biol. Chem.*, 1930, lxxxviii, 743. The Determination of Peptic Activity: an Examination and Application of the Gates Method of Proteolytic Enzyme Titration.

- 3 Hirsch-Mammoth, Paul and Rindfleisch Hermann, Deutsche med Wehnschr , 1925, 1, 512 Ergebnisse der Magensaftuntersuchung mit der neuen Pepsinbestimmung nach Boas
- 4 Polland, W S, and Bloomfield A L, J Clin Invest 1930, ix, 107 The Diagnostic Value of Determinations of Pepsin in Gastric Juice
- 5 Wilcox, W H, Lancet, 1908, ii, 220 The Importance and Significance of the Chemical Examination of the Gastric Contents after a Test Meal with a New Method for Estimating the Ferment Activity of the Gastric Contents
- 6 Osterberg, A E, Vanzant F R and Alvarez, W C, J Clin Invest, 1933, xii, 551 Studies of Gastric Pepsin I Methods of Measurement and Factors which Influence It

STUDIES OF UREA EXCRETION VIII THE EFFECTS ON THE UREA CLEARANCE OF CHANGES IN PROTEIN AND SALT CONTENTS OF THE DIET

By CUTHBERT L. COPE¹

(From the Hospital of the Rockefeller Institute for Medical Research, New York)

(Received for publication January 14 1933)

In the study of the effects of protein rich and protein poor diets on nephritic kidneys, relatively little attention has so far been paid to the changes in functional activity. Indications suggesting that such changes exist are to be found in the observations of Addis and Drury (1) that the "maximum urea clearance" can be slightly but definitely increased by a meal of protein-rich food, and in the recently published findings of Jolliffe and Smith (2) that in dogs fed on a cracker meal diet low in protein, the urea clearance is markedly lower than on a high protein diet.

It appeared desirable, therefore, to obtain more definite information concerning the effects of protein on the function of human nephritic kidneys. As criterion of renal function, the standard urea clearance of Möller, McIntosh and Van Slyke (5) has been employed. The cases presented are unselected, except that those were discarded which showed evidence during the preliminary observational period that activity of the nephritic process was causing a progressive lowering of renal function at the time, and also a few in which infections or exacerbations occurred during the experimental period and were likely to change the renal function. These two types of cases were excluded because they were subject to such rapid changes that it would have been difficult to tell whether functional changes under different regimes were due to change in regime or in renal pathology. The patients used were those in which the renal condition appeared to be in a fairly steady state.

In order to obtain evidence of the effect of salt also on the urea clearance, similar studies were carried out with diets containing large and minimal amounts of sodium chloride. The salt variation had no significant influence on the urea clearance. We shall therefore not detail this part of the work, but merely record its negative results. In one instance the ingestion of 65 grams of sodium chloride and its subsequent excretion at high concentration in the urine had no detectable influence on the urea clearance. It was not found possible, by feeding diets poor in salt, to reduce the plasma chloride to such low concentrations as are

¹ Beit Memorial Research Fellow

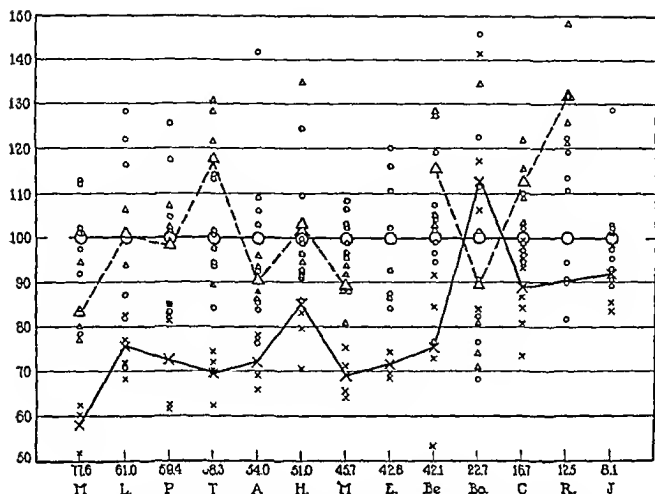


FIG 1 EACH COLUMN OF SYMBOLS SHOWS STANDARD UREA CLEARANCE VALUES FOR AN INDIVIDUAL WHOSE MEAN STANDARD UREA CLEARANCE ON NORMAL DIET (ABOUT 75 GRAMS PROTEIN PER DAY) IS RECORDED ALONG THE LOWER MARGIN

Ordinates represent clearance values expressed for each subject as percentages of his average clearance on a diet with 75 grams of protein

- = values obtained on normal diet (75 grams protein per day),
- = mean of these values,
- × = values obtained on low protein diet (40 grams protein per day),
- × = mean of these values,
- △ = values obtained on high protein diet (120 grams protein per day),
- △ = mean of these values

per minute, as shown by the small crosses. When the daily protein intake was increased to 120 grams the standard clearance rose once more to be between 77 per cent and 94 per cent of the original mean, i.e. to between 59.7 and 73.0 cc per minute, the individual clearances being indicated by the small triangles.

The figure shows that when renal function is unimpaired, or only slightly impaired by the nephritic lesion, as in the first 9 cases, the urea clearance is reduced by from 15 to 40 per cent by diminishing the protein intake from 75 grams to 40 grams per day. Of the remaining cases, with seriously impaired renal functions, two showed a slight reduction of the urea clearance, about 10 per cent, and one was exceptional in having a urea clearance higher on the low protein than on a protein rich diet. No

said by many French writers to cause a nitrogen retention. Diets which reduced the urinary NaCl output to less than 1 gram per day, however, were without effect on the urea clearance.

METHODS

The usual methods of estimating and calculating the clearance have been employed (5). Urea in both blood and urine has been estimated gasometrically by the method of Van Slyke (7), in the former in 1 cc samples of whole blood obtained by venipuncture, in the latter in a volume of urine appropriate to the urea concentration anticipated. Duplicate analyses made in all cases agreed within 1 per cent.

After admission to hospital and routine investigation each patient was kept for a week or more on a normal diet containing about 75 grams protein per day. During this time urea clearance determinations, usually eight in number, were made with the patient in bed, for the dual purpose of obtaining average values for the individual on a normal diet, and of making sure that no marked spontaneous change in the urea clearance was taking place. A diet containing 40 grams of protein per day was then given for at least five days before fresh clearance determinations were made. These having been obtained, the protein of the diet was once more increased either to the original 75, or in most cases to 120, grams per day. Rise of the urea clearance during this third period in each of the recorded cases (except the anomalous Bo) was taken as evidence that the depressed clearance in the second period was indeed associated with the low protein diet, and was not an expression of a progressively destructive nephritic process active at the time.

RESULTS

Thirteen cases of nephritis have been successfully examined in this manner, and the results are indicated graphically in Figure 1. All the clearance values obtained on a given individual are plotted in a single column. The thirteen columns thus represent the thirteen cases studied and are arranged in order of decreasing renal function. The figure at the bottom of each column is the mean standard urea clearance for that individual on a normal diet, expressed in cubic centimeters of blood per minute. The individual clearance values obtained have been plotted as percentages of this figure. Thus in each case an ordinate value of 100 per cent represents the average standard urea clearance for that subject on a normal diet. To take an example, the first column represents observations on the patient M. On a normal diet his mean standard urea clearance was 77.6 cc per minute. Individual clearance determinations, plotted as small circles, varied from 112 per cent to 79 per cent of *this value*. On a low protein diet the urea clearance fell to between 51 per cent and 62.5 per cent of the original 77.6 cc, i. e. to between 39.6 and 48.5 cc

rise which followed urea ingestion, and was falling, and that the amount of urea formed from the food or glutamic acid was insufficient to check the fall in blood urea. It appears probable, therefore, that the stimulus to increased blood urea clearance was something other than urea itself.

In this connection it is of interest that the hypertrophy of the kidneys, which many workers have produced by feeding a high protein diet, could not be fully duplicated by Osborne, Mendel, Park and Winternitz (6) nor by MacKay, MacKay and Addis (4), by feeding urea alone, a fact which tends to suggest that such hypertrophy also is related to the stimulating effect of products other than urea formed from a protein diet.

There is, it is true, one gap in the experimental evidence required to confirm the application of this explanation to our results. All the data available on men have been obtained by starting with subjects in ordinary protein nutrition, with the usual blood urea nitrogen levels of 10 to 16 milligrams per 100 cc. To obtain by urea administration the same blood urea changes that are produced by increasing the protein intake from 40 grams to 75 or more grams, one would need to use subjects who had previously been on a diet containing as little as 40 grams of protein. If urea administration to such subjects did not stimulate a rise in the clearance, it would appear clear that the increased clearances obtained in our subjects by increased protein administration were not due to the accompanying rise in blood urea.

SUMMARY

The urea clearance was not affected, in either normal or nephritic subjects, by variations in the sodium chloride content of the diet.

Raising the protein content of the diet from 75 grams to 120 grams was also without consistent effect on the urea clearance.

However, lowering the protein intake from 75 grams to 40 grams was accompanied by depression of the clearance in 11 out of 12 subjects *with normal or nearly normal renal function*. In these subjects the clearance on the low protein diet was only 60 to 80 per cent as great as on the 75 gram diet.

In three patients with renal function *below half normal*, the clearance on low protein remained 90 per cent or more of the clearance on the 75 gram diet.

In interpreting subnormal urea clearances as measures of renal function, allowance should be made for the fact that on very low protein diets the lower limit of normal variation may extend down to 50 per cent of the usual normal average, instead of to the 70 per cent minimum found under ordinary conditions (5). In subjects with markedly depressed function, however, the clearance appears to be relatively fixed, and little influenced by protein restriction.

explanation of this anomalous case is offered. In the remaining case, that of R, the low protein diet was not administered, as her plasma proteins were low and it was felt that such a regime would provoke a recurrence of the edema to which she was liable. This case is included, however, because she showed a well marked rise in her urea clearance when the protein intake was increased to 120 grams per day.

The effect of high protein regime was more irregular than that of the low. Of the eleven cases which had the high protein intake during the third period, the clearances of four rose to well above the figures for the first or moderate (75 grams) protein diet period, those of three others returned to the original values, and those of a further three rose well above the low protein diet figures, but failed to return completely to the original level. The remaining case Bo was again anomalous showing an effect precisely the reverse of the other twelve, her clearance being reduced on the high protein diet.

DISCUSSION

In 11 out of the 12 nephritic patients who have been examined a definite reduction in the urea excretory activity of the kidneys has been demonstrable on a low protein diet, when this activity has been estimated by the standard urea clearance test.

In this respect our data confirm the observations of Keutmann and McCann (3) which appeared when our experimental work was nearing completion. Keutmann and McCann studied 4 cases of hemorrhagic Bright's disease, and found that the urea clearance was usually higher on high protein than on low protein diets.

In our cases, the effect of low protein diet in reducing the urea clearance is relatively less when the renal activity is already seriously impaired by disease. This fact is in accord with other evidences that the kidney shows a tendency to diminishing variability of activity as the nephritic lesion progresses. Possibly in this instance it is due to retained metabolites stimulating the kidney to a sustained maximal effort.

In all cases the rise in urea clearance caused by increasing the protein intake was accompanied by a rise in the blood urea concentration. However, it appears probable that the kidneys are stimulated by some protein metabolite other than urea. Moller, McIntosh, and Van Slyke (5) found that, when the urea nitrogen content of the blood was increased from the usual normal range of 10 to 16 mgm per 100 cc to two or three times as much by feeding urea, the clearance value was not affected. Addis and Drury (1) found that, when the blood urea was first similarly raised by urea ingestion, and later a protein meal or 20 grams of glutamic acid was taken, the meal or amino acid caused a definite decrease in the clearance. The increase in clearance after food or glutamic acid occurred despite the facts that the blood urea had some hours before passed the peak of the

rise which followed urea ingestion, and was falling, and that the amount of urea formed from the food or glutamic acid was insufficient to check the fall in blood urea. It appears probable, therefore, that the stimulus to increased blood urea clearance was something other than urea itself.

In this connection it is of interest that the hypertrophy of the kidneys, which many workers have produced by feeding a high protein diet, could not be fully duplicated by Osborne, Mendel, Park and Winternitz (6) nor by MacKay, MacKay and Addis (4), by feeding urea alone, a fact which tends to suggest that such hypertrophy also is related to the stimulating effect of products other than urea formed from a protein diet.

There is, it is true, one gap in the experimental evidence required to confirm the application of this explanation to our results. All the data available on men have been obtained by starting with subjects in ordinary protein nutrition, with the usual blood urea nitrogen levels of 10 to 16 milligrams per 100 cc. To obtain by urea administration the same blood urea changes that are produced by increasing the protein intake from 40 grams to 75 or more grams, one would need to use subjects who had previously been on a diet containing as little as 40 grams of protein. If urea administration to such subjects did not stimulate a rise in the clearance, it would appear clear that the increased clearances obtained in our subjects by increased protein administration were not due to the accompanying rise in blood urea.

SUMMARY

The urea clearance was not affected, in either normal or nephritic subjects, by variations in the sodium chloride content of the diet.

Raising the protein content of the diet from 75 grams to 120 grams was also without consistent effect on the urea clearance.

However, lowering the protein intake from 75 grams to 40 grams was accompanied by depression of the clearance in 11 out of 12 subjects *with normal or nearly normal renal function*. In these subjects the clearance on the low protein diet was only 60 to 80 per cent as great as on the 75 gram diet.

In three patients with renal function *below half normal*, the clearance on low protein remained 90 per cent or more of the clearance on the 75 gram diet.

In interpreting subnormal urea clearances as measures of renal function, allowance should be made for the fact that on very low protein diets the lower limit of normal variation may extend down to 50 per cent of the usual normal average, instead of to the 70 per cent minimum found under ordinary conditions (5). In subjects with markedly depressed function, however, the clearance appears to be relatively fixed, and little influenced by protein restriction.

BIBLIOGRAPHY

- 1 Addis, T, and Drury, D R, J Biol Chem, 1923, lv, 629 The Rate of Urea Excretion VII The Effect of Various Other Factors than Blood Urea Concentration on the Rate of Urea Excretion
- 2 Jolliffe, N, and Smith, Homer, W, Am J Physiol, 1931, xcix, 101 The Excretion of Urine in the Dog II The Urea and Creatinine Clearance on Cracker Meal Diet
- 3 Keutmann, E H, and McCann, W S, J Clin Invest, 1932, xi, 973 Dietary Protein in Hemorrhagic Bright's Disease I Effects upon the Course of the Disease with Special Reference to Hematuria and Renal Function
- 4 MacKay, L L, MacKay, E M, and Addis, T, J Nutrition, 1931, iv, 379 Factors Which Determine Renal Weight XII The Nitrogen Intake as Varied by the Addition of Urea to the Diet
- 5 Moller, E, McIntosh, J F, and Van Slyke, D D, J Clin Invest, 1928, vi, 427 Studies of Urea Excretion II Relationship between Urine Volume and the Rate of Urea Excretion by Normal Adults
- 6 Osborne, T B, Mendel, L B, Park, E A, and Winternitz, M C, J Biol Chem, 1927, lxxi, 317 Physiological Effects of Diets Unusually Rich in Protein or Inorganic Salts
- 7 Van Slyke, D D, J Biol Chem, 1927, lxxiii, 695 Determination of Urea by Gasometric Measurement of the Carbon Dioxide Formed by the Action of Urease

THERMAL CHANGES IN PERIPHERAL VASCULAR DISEASE DURING SYMPATHETIC GANGLIONECTOMY UNDER GENERAL ANESTHESIA

BY WINCHELL McK CRAIG, BAYARD T HORTON
AND CHARLES SHEARD

*(From the Section on Neurologic Surgery and the Division of Medicine The Mayo Clinic
and the Division of Physics and Biophysical Research
The Mayo Foundation Rochester, Minn)*

(Received for publication February 2 1933)

A great deal of confusion has existed regarding the differential diagnosis of Raynaud's disease, thrombo angitis obliterans, and arteriosclerosis. Raynaud's disease affects, predominantly, young women, and because it is caused by vasospasm is completely relieved in the uncomplicated case, by sympathetic ganglionectomy. It need not be confused with thrombo angitis obliterans and arteriosclerosis with occlusion. Arteriosclerosis with occlusion is usually found among elderly people, and since vasospasm is rarely, if ever, an element in the condition, it is not affected by sympathetic ganglionectomy. Thrombo angitis obliterans practically always affects young men, has an element of vasospasm, and many of the patients can be satisfactorily treated by sympathetic ganglionectomy. Simple tests have been sought which would aid in differential diagnosis and which, by revealing the degree of vasodilation possible, would help to determine the suitability of these cases for operation, and, in some degree, indicate the probable prognosis. Observations made on patients undergoing sympathetic ganglionectomy under general anesthesia revealed certain thermal changes which may prove of value in this connection.

With the use of general anesthesia, administered by inhalation, it was noted that extremities which were not denervated were temporarily relieved of vasomotor spasm, and that there was definite postoperative clinical improvement of these extremities. It was difficult to interpret this temporary improvement, but it was thought for a time that the entire sympathetic nervous system was reflexly or indirectly affected by operation on, or manipulation of, any part. Adson (1) ventured the suggestion that the general anesthetic probably influenced the entire sympathetic nervous system by affecting the vasoconstrictor center in the diencephalon. Morton and Scott (6) measured the changes in cutaneous

¹ Read before the Central Society for Clinical Research, Chicago, Illinois
November 4 1932

temperature caused by different anesthetic agents and called attention to the different temperature gradients in the extremities of normal people. They suggested the use of general anesthesia as a means of selecting cases suitable for sympathetic ganglionectomy, but concluded that it was not satisfactory. Sheard, Ryneerson and Craig (9) have shown experimentally that in dogs under anesthesia by inhalation, generalized vasodilation occurred and that this was not influenced by operation on the sympathetic system. Also they showed that under general anesthesia the changes in the temperatures of intact and of sympathectomized extremities, with variations in environmental temperature, were similar.

Herrick, Essex and Baldes (5), using a modification of the thermomuhr method of Rein, have shown that following unilateral sympathetic ganglionectomy on dogs, the flow in the femoral arteries on the sympathectomized side was about twice as great as that on the intact side. There was no outstanding difference between the readings of the two sides when, following the operations, these animals were placed under ether anesthesia. Hence, it may be concluded that during surgical anesthesia by ether, there develops vasodilation of the vessels of the extremities as great as that following sympathetic ganglionectomy.

Thus, it would seem that during operations on the sympathetic nervous system under anesthesia by inhalation, there occurred certain physiologic changes which could be traced directly to the anesthetic, and which were not altered by removal of ganglia. By observing the changes in cutaneous temperature during operations under general anesthesia, certain important facts were obtained which we believe will prove of diagnostic and prognostic value in treating cases of peripheral vascular disease.

This study consisted of continuous observations of surface temperature of the extremities of subjects with Raynaud's disease and thrombo-angitis obliterans during the course of thoracic and lumbar sympathetic ganglionectomy. Repeated readings of temperature were made preoperatively with the subjects at rest and with the room temperature at approximately 24° C. A portable electromotive thermometer, which is a modification of the ensemble described by Sheard (8), was used throughout the investigations. The instrument used carried four thermocouples for application to the hands and feet. By means of a rotary switch, any thermocouple could be placed in the circuit and the temperature indicated by each of the thermocouples could be read quickly and accurately on the micro-ammeter calibrated in degrees Centigrade. The thermocouples were left in place during the entire course of the operation, so that graphic records were obtained of the changes in surface temperature of the extremities as influenced by anesthesia alone and by the interruption of the sympathetic innervation to the extremities. The operations were carried out under general anesthesia with nitrous oxide, oxygen and ether, or ethylene, oxygen and ether. Regardless of induction and maintenance

with gas, oxygen, or ether administered by the drop method, the relative changes in the temperature curve were the same. Observations of the blood pressure and pulse were made during this period. For a control study, similar observations of changes of surface temperature were made on subjects during the course of operation for other conditions.

The rise in surface temperature of the extremities of an apparently normal subject is illustrated in Figure 1 and represents a normal response

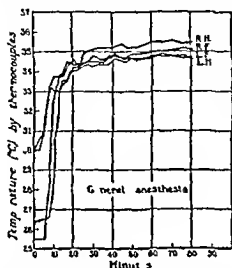


FIG 1 COURSE OF THERMAL CHANGES IN HANDS AND FEET OF A PATIENT WITH APPARENTLY NORMAL VASOMOTOR REACTION TO GENERAL ANESTHESIA

R H, right hand R F, right foot L F, left foot, and L H, left hand

of vasodilation. There was a relatively sharp and uniform rise in the surface temperature of all four extremities, within the first ten minutes after the induction of anesthesia. The maximal rise of surface temperature occurred within twenty to thirty minutes and remained constant for one hour, the pulse rate varied from 70 to 80 beats each minute, and the systolic blood pressure varied from 122 to 130 mm Hg and the diastolic, from 60 to 74 mm Hg.

The marked similarity between the rise in surface temperature of the extremities in a subject with Raynaud's disease and that of the normal person is illustrated by Figure 2a. The same sharp rise in surface temperature occurred in all four extremities immediately after induction of anesthesia, and reached its maximum within twenty to twenty five minutes. Practically no change was observed in the surface temperature of the extremities after this period. This is what one would expect in a case of Raynaud's disease, which is a primary vasospastic disturbance involving the vessels of the extremities. It is interesting to note that no change in the surface temperature of the lower extremities was observed following interruption of the sympathetic nerve supply. In other words, the maximal rise in surface temperature had occurred immediately following induction of the anesthesia, and severance of the sympathetic nerve supply caused no additional rise in surface temperature. This is of particular interest because it was formerly a clinical impression among certain

workers that immediately following interruption of the sympathetic nerve supply to a given extremity, there was always a sharp rise of surface temperature and an increase in flow of blood in that extremity

The rise in surface temperature of the extremities of subjects with thrombo-angitis obliterans varied considerably among different persons

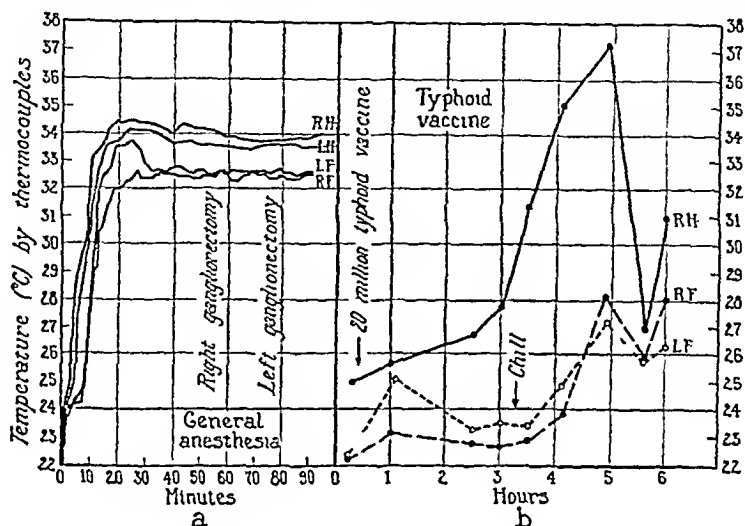


FIG 2 INCREASE IN SURFACE TEMPERATURE OF THE HANDS AND FEET OF A WOMAN AGED TWENTY-THREE YEARS WHO HAD RAYNAUD'S DISEASE

Pulsations in the peripheral vessels were normal *a* The rise with general anesthesia *b* The rise obtained with intravenous injection of typhoid vaccine Rise in mouth temperature was 1.1°C R H, right hand, R F, right foot, L F, left foot, and L H, left hand

and corresponded clinically to the degree of occlusive vascular disease present (Figs 3a, 4a, 5a and 6a) In thrombo-angitis obliterans, one is dealing primarily with an occlusive vascular disease which involves the extremities Vasospastic disturbance in the collateral circulation is invariably present to some degree Interruption of the sympathetic nerve supply to the affected extremities permits the maximal flow of blood through the collateral circulation, but it is obvious that the occluded vessels are not affected by this surgical procedure In cases in which there is a small degree of occlusion and a large degree of superimposed vasospasm in the collateral circulation, the rise in surface temperature of the extremities parallels that observed in cases of Raynaud's disease In moderately advanced cases of thrombo-angitis obliterans, uniform rises in surface temperature of all four extremities were not observed, but in early cases the rise in surface temperature of the extremities may closely simulate that observed in Raynaud's disease, as illustrated in Figure 3a

It is interesting to compare the various rises in temperature obtained with intravenous administration of typhoid vaccine in a given case and

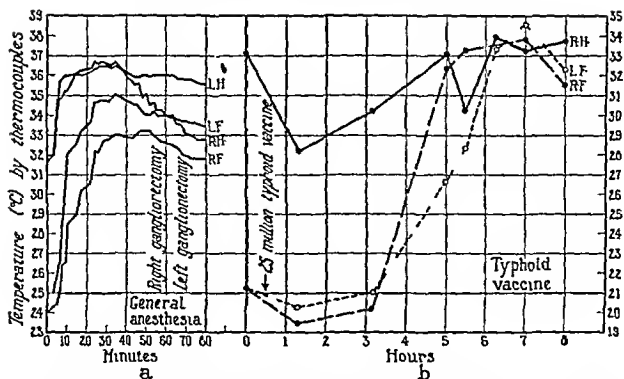


FIG 3 VARIATION IN SURFACE TEMPERATURES OF THE HANDS AND FEET OF A MAN AGED THIRTY TWO YEARS WHO HAD THROMBO ANGIITIS OBLITERANS

Pulsations in the right and left ulnar, radial femoral and popliteal arteries were normal. In other arteries pulsations were reduced as follows: in the right dorsalis pedis, 80 per cent, in the left dorsalis pedis, 65 per cent, in the right posterior tibial, 35 per cent, and in the left posterior tibial 25 per cent. *a* The rise with general anesthesia. *b* The rise obtained with intravenous injection of typhoid vaccine. Rise in mouth temperature was 0.5°C . R H, right hand; R F, right foot; L F, left foot, and L H, left hand.

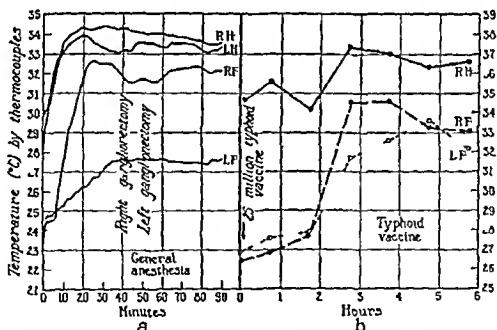


FIG 4 INCREASE IN SURFACE TEMPERATURE OF THE HANDS AND FEET OF A MAN AGED FORTY YEARS WHO HAD THROMBO ANGIITIS OBLITERANS

The right and left ulnar, radial and femoral arteries pulsated normally. Pulsations in both popliteal arteries were reduced approximately 50 per cent. In the right and left dorsalis pedis and posterior tibial arteries pulsations were absent. Circulatory insufficiency was more marked in the left than in the right foot. *a* The rise with general anesthesia. *b* The rise obtained with intravenous injection of typhoid vaccine. Rise in mouth temperature was 1.3°C . R H, right hand; R F, right foot; L F, left foot, and L H, left hand.

that obtained following induction of general anesthesia. We have previously been of the opinion that similar temperature curves could be obtained. This, however, proved not to be true, for there was marked dissimilarity between the curves obtained following intravenous administration of typhoid vaccine and that following induction of general anesthesia (Figs 2b, 3b, 4b, 5b and 6b). This in part may be due to the fact that some subjects are either refractory to or react poorly to intravenous administration of typhoid vaccine. The maximal flow of blood to the extremities occurred following both procedures, but it occurred more quickly after general anesthesia than after intravenous administration of typhoid vaccine. With general anesthesia, fewer fluctuations were observed after the surface temperature had reached its maximum. It

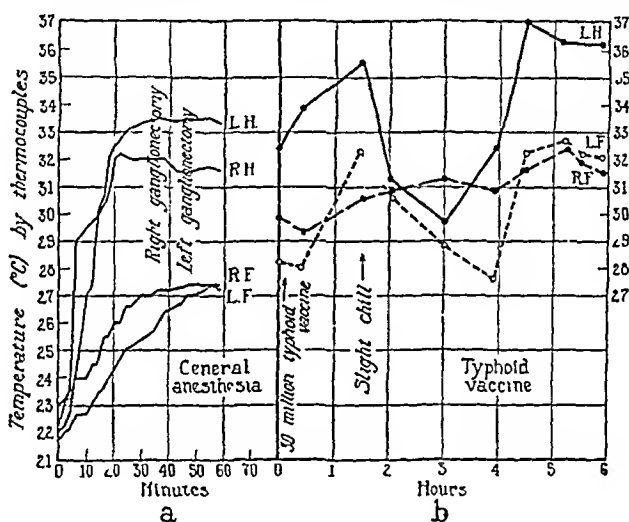


FIG 5 VARIATION IN SURFACE TEMPERATURE OF THE HANDS AND FEET OF A MAN AGED TWENTY-THREE YEARS WHO HAD THROMBO-ANGIITIS OBLITERANS

Pulsations in the right and left ulnar, radial, and femoral arteries, and in the right popliteal arteries, were normal, those in the left popliteal artery were reduced 25 per cent. Pulsations in the right and left dorsalis pedis arteries were reduced 40 per cent, and the right and left posterior tibial arteries were occluded. The circulatory insufficiency was the same in both feet. *a* The rise with general anesthesia. *b* The rise obtained with intravenous injection of typhoid vaccine. Rise in mouth temperature was 2.2°C. R H, right hand, R F, right foot, L F, left foot, and L H, left hand.

was also evident that the maximal flow of blood to the extremities following general anesthesia occurred within twenty or thirty minutes and was not increased after that time. The fluctuation in the curves of surface temperature following intravenous injection of typhoid vaccine is accounted for, in part at least, by the chill which so frequently develops. Brown originally used typhoid vaccine intravenously to determine what he designated "the vasomotor index." He and Adson (2, 3) used this

method for selecting subjects with Raynaud's disease and thrombo-angitis obliterans for sympathetic ganglionectomy. It has proved to be a satisfactory means of selecting cases for this surgical procedure. In addition, the intravenous administration of typhoid vaccine or "fever therapy" undoubtedly represents the most important medical method of treatment for these diseases. This is particularly true with reference to thrombo-angitis obliterans.

Other methods for selecting cases for sympathetic ganglionectomy are spinal anesthesia, which is applicable only to the lower extremities, and paravertebral injection of procaine in the cervicothoracic region for the upper extremities. Oral administration of alcohol, intramuscular injection

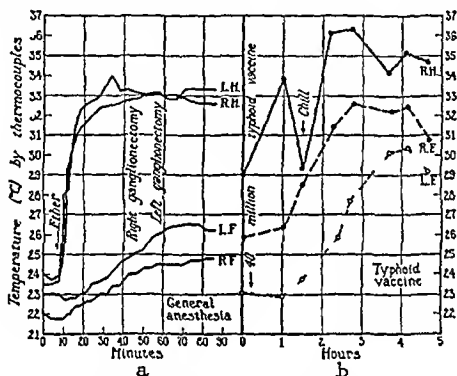


FIG 6 VARIATION IN SURFACE TEMPERATURE OF HANDS AND FEET OF A MAN AGED THIRTY TWO YEARS WHO HAD THROMBO ANGIITIS OBLITERANS

Pulsations in the right ulnar, radial, and femoral arteries, and in the left radial and femoral arteries, were normal. Pulsations were absent in the other palpable vessels. Circulatory insufficiency was more marked in the right than in the left foot. *a* The rise with general anesthesia. *b* The rise obtained with intravenous injection of typhoid vaccine. Rise in mouth temperature was 1°C. R H, right hand; R F, right foot; L F, left foot, and L H, left hand.

tion of acetylcholine, application of heat, and many other procedures also have been used with varying degrees of success. The procedure of observing surface temperatures of the subject under anesthesia affords a satisfactory method for investigating the amount and extent of vasospastic disturbances particularly in the extremities of subjects with Raynaud's disease and thrombo-angitis obliterans. If satisfactory rises in the surface temperature of the involved extremities are not observed after induction of anesthesia, it is evident that an additional rise will not develop after interruption of the sympathetic innervation. The

observations were made in investigating cases of vasospastic scleroderma and chronic infectious arthritis. Thermal changes under general anesthesia give valuable information from the prognostic standpoint in subjects with thrombo-angitis obliterans. Accurate information can be obtained regarding the extent of the occlusive process in the extremities and the amount of superimposed vasospasm in the collateral circulation. With the history of the case, the physical examination, and the information derived from such a study, the prognosis in a given case can be estimated fairly adequately.

SUMMARY AND CONCLUSIONS

Continuous observations of surface temperature, before and during sympathetic ganglionectomy, were made on all four extremities of subjects with Raynaud's disease and thrombo-angitis obliterans. An electromotive thermometer was used. The difference between these two diseases is well illustrated by this study. General anesthesia alone produced maximal vasodilation in Raynaud's disease and thrombo-angitis obliterans. Severance of the sympathetic nerves did not cause additional vasodilation. In Raynaud's disease a prompt and uniform vasodilating response was observed in the peripheral vessels of all four extremities, but in thrombo-angitis obliterans uniform response was absent because the occlusive process in the vessels of all four extremities is never the same in thrombo-angitis obliterans.

The graphic records made with anesthesia at the time of operation serve as a check on preoperative studies, carried out with other vasodilating agents. If satisfactory rises in the surface temperature of the involved extremities are not observed after induction of general anesthesia, it is evident that an additional rise will not develop after interruption of the sympathetic innervation.

General anesthesia constitutes a satisfactory method for investigating thermal changes in cases of thrombo-angitis obliterans, Raynaud's disease, vasospastic scleroderma, and arthritis, prior to and during sympathetic ganglionectomy. Valuable information may be obtained regarding the amount and extent of vasospasm in the vessels of the extremities, particularly in the collateral circulation of subjects with thrombo-angitis obliterans, and the method gives accurate information regarding the degree of occlusive process in this group of cases. Vessels of patients who are refractory to, or react poorly to, other vasodilating agents will usually undergo maximal vasodilation when general anesthesia is administered.

BIBLIOGRAPHY

- 1 Adson, A. W., Personal communication to the authors.
- 2 Brown, G. E. and Adson, A. W., *Am J M Sc*, 1925, clxv, 232. Calorimetric Studies of the Extremities Following Lumbar Sympathetic Ramisection and Ganglionectomy.

- 3 Brown, G E and Adson, A W, Arch Neurol and Psychiat, 1929, xxii 322
Physiologic Effects of Thoracic and of Lumbar Sympathetic Ganglionectomy or Section of the Trunk
- 4 Herrick, Julia F and Baldes, E J, Physics, 1931, 1, 407 The Thermo Stromuhr Method of Measuring Blood Flow
- 5 Herrick, J F, Essex, H E and Baldes E J, Am J Physiol, 1932, ci, 213
The Effect of Lumbar Sympathectomy on the Flow of Blood in the Femoral Artery of the Dog
- 6 Morton, J J and Scott, W J M, J Clin Invest, 1930, ix, 235 The Measurement of Sympathetic Vasoconstrictor Activity in the Lower Extremities
- 7 Royle, N D, M J Australia, 1924, 1, 77 A New Operative Procedure in the Treatment of Spastic Paralysis and its Experimental Basis
- 8 Sheard, Charles, Am J Clin Path, 1931, 1, 209 The Electromotive Thermometer An Instrument and a Method for Measuring Intramural, Intravenous, Superficial and Cavity Temperatures
- 9 Sheard, Charles Rynearson E H and Craig, W McK, J Clin Invest, 1932, xi 183 Effects of Environmental Temperature, Anesthesia and Lumbar Sympathetic Ganglionectomy on the Temperatures of the Extremities of Animals

GLYCOLYSIS IN THE BLOOD OF PATIENTS WITH PERNICIOUS ANEMIA

By S MILTON GOLDHAMER

*(From the Thomas Henry Simpson Memorial Institute for Medical Research University
of Michigan, Ann Arbor)*

(Received for publication February 7, 1933)

Following the discovery by Warburg that tumor cells have quantitative and qualitative differences from those of normal tissues with regard to their carbohydrate metabolism, many investigators have demonstrated that the method of glycolysis was most satisfactory for the study of the metabolism of the blood cells. Maclean and Weir (1) pointed out the role played by the different blood elements in glycolysis, and showed that the erythrocytes were a vital factor in the sugar consumption of normal blood. Inasmuch as the red blood cells play an important part in the glycolytic activity of normal blood, and since pernicious anemia is a disease primarily involving the red cells (both number and type), a study of the glycolysis of the blood in this malady has been undertaken for further investigation of the metabolism of the red cells.

METHODS

Blood sugar determinations were made from the blood of nine patients with pernicious anemia before treatment and at the height of the reticulocyte response, following ventriculin therapy. Red cell, white cell and reticulocyte counts were made on corresponding days.

The blood sugar values were calculated according to the micro method of Folin and Wu (2). All the estimations were made from blood samples obtained before breakfast at 8 A M. The usual procedure was to withdraw 10 to 15 cc. of blood from the arm vein under sterile precautions, no tourniquet being used in order to avoid stasis. The blood was discharged into a large test tube, mixed with heparin, and kept in a warm room at a temperature of 37° C. Heparin was used as an anti coagulant as it was shown by Falcon Lesses (3) to have no appreciable effect on the rate of glycolysis.

The blood sugar content was determined immediately after the removal of the specimen from the patient, and then at hourly intervals for 5 hours to estimate the glycolytic activity. The red blood cell and white blood cell counts were made with U S Bureau of Standards pipettes and Neubauer-Levy counting chambers. Reticulocyte estimations were made from brilliant cresyl blue film preparations.

TABLE I
Rate of glycolysis in pernicious anemia during relapse

Case number	Red blood cells millions per cu mm	White blood cell count	Reticulo-cyte per cent	Mgm glucose per 100 cc blood						Rate of glycolysis in mgm glucose per 100 cc blood per hour					
				Initial values	Values after					1st hour	2d hour	3d hour	4th hour	5th hour	Average per hour
					1 hour	2 hours	3 hours	4 hours	5 hours						
1	1.98	8400	2.0	137	120	109	95	79	70	17	11	14	16	9	13.4
2	2.90	5500	0.5	94	87	80	74	68	58	7	7	6	6	10	7.2
3	1.80	7450	3.1	78	67	62	58	55	47	9	5	4	3	8	5.8
4	2.27	5600	0.7	86	75	65	60	55	53	11	10	5	5	2	6.6
5	1.20	5000	3.7	104	92	91	83	80	79	12	1	8	3	1	5.0
6	1.38	2550	1.8	107	95	90	83	79	75	12	5	7	4	4	6.4
7	1.81	5600	0.8	130	121	108	100	100	93	9	13	8	0	7	7.4
8	.83	4900	1.2	120	120	117	115	111	107	0	3	2	4	4	2.8
9	1.27	3650	1.6	115	113	109	107	104	101	2	4	2	3	3	2.8
Average	1.71	5405	1.7	108	99	92	86	81	76	9	7	6	5	5	6.4

DATA

In Table I are presented the results of the glucose determinations of the blood from 9 cases with pernicious anemia during relapse. The average initial glucose value is 108.0 mgm per 100 cc blood. There is a uniform rate of glycolysis averaging about 6.4 mgm per hour, the limits being 5 to 9 mgm. The average rate of glycolysis per hour per million red cells is about 3.7 mgm, which is approximately the same as in normal blood.

In Table II are listed the results of the blood sugar determinations of 9 patients with pernicious anemia at the height of the reticulocyte response following ventriculin therapy. The initial average glucose value is 100 mgm per 100 cc blood. The average rate of glycolysis per hour is 10.2 mgm, the extremes being 8 to 13 mgm. The average rate of glycolysis per hour per million red cells is 5.4 mgm. These averages are about 65 per cent more than the glycolytic rate before treatment.

Compared to the standard rate of glycolysis of normal blood as established by Schmitz and Glover (4), and Falcon Lesses (3), the glycolytic activity of the blood in pernicious anemia during relapse or early remission is markedly retarded. In Chart 1 is a comparison of the average rate of glycolysis of 9 cases with pernicious anemia during relapse and in early remission with normal glycolytic activity as determined by Schmitz and Glover, and Falcon Lesses.

Although the initial average glucose values in the cases of pernicious anemia were slightly elevated, they remained within the upper limits of normal. Many investigators have pointed out the fact that glycolysis proceeds at a faster rate with a high initial glucose concentration. In view of this, then, the rate of glycolysis in pernicious anemia would be even slower by comparison, since the initial glucose values are higher than those of normal blood. It is further noted that the initial values of the blood sugar determinations of patients with pernicious anemia in early remission are less than those of the patients in relapse, yet the average rate of glycolysis in the former cases is about 65 per cent more. In all instances, glycolysis appears to progress at a fairly uniform rate, averaging 15 to 17 mgm per hour in the normal, 5 to 9 mgm per hour in pernicious anemia during relapse (red blood cell counts from 0.83 to 2.90 millions per cu mm), and 8 to 13 mgm per hour in pernicious anemia during early remission (red blood cell counts from 0.80 to 2.90 millions per cu mm).

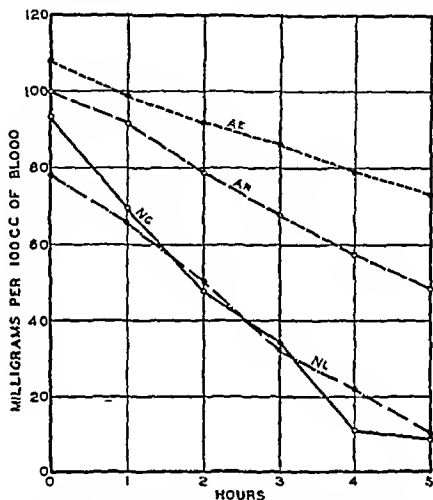
Calculated from figures for normal, the average rate of glycolysis per hour per million red blood cells is about 3.5 mgm. In pernicious anemia, the average rate of glycolysis per hour per million red blood cells is 3.7 mgm. The decreased rate of glycolysis noted in the blood in anemia as compared to that in the normal blood was definitely proportionate to the decreased number of red blood cells. In the cases of pernicious ane-

TABLE II
Rate of glycolysis in pernicious anemia in early remission following ventriculin therapy

Case number	Red blood cells	White blood cell count	Reticulo cyte	Mgm glucose per 100 cc. blood						Rate of glycolysis in mgm glucose per 100 cc. blood per hour					
				Initial values	Values after					1st hour	2d hour	3d hour	4th hour	5th hour	Average per hour
					1 hour	2 hours	3 hours	4 hours	5 hours						
	millions per cu mm		per cent												
1	2 13	12500	15 5	111	104	72	61	42	31	7	32	11	19	11	16
2	2 90	5000	6 0	85	76	68	59	50	44	9	8	9	9	6	8 2
3	2 25	6950	15 7	81	72	63	55	45	37	9	9	8	10	8	8 8
4			16 5	92	87	71	51	43	30*	5	16	20	8	13	11 8
5	1 80	5500	22 0	108	89	70	52	40	30*	19	19	18	12	10	15 6
6	1 30	4650	20 0	102	88		76	71	66	14			5	5	8 0
7	2 10	2750	12 5	102	93	87	79	68	58	9	6	8	11	10	8 8
8	80	5500	42 0	121	120	108	99	89	80	1	12	9	10	9	8 2
9	1 60	9500	15 7	102	97	91	83	74	66	5	6	8	9	8	7 2
Average	1 86	6544	18 4	100	92	79	68	58	49	8	13	11	10	9	10 2

* Less than 30 mgm

mia in early remission, the blood presents, in addition to a decreased number of red cells, a marked increase in the number and percentage of immature red cells or reticulocytes. The average rate of glycolysis per hour per million red blood cells in this instance is 5.4 mgm. Compared to the rate of glycolysis in normal blood there is a decrease proportional to the decreased number of red cells present. However, in comparison to the rate of glycolysis per hour per million red blood cells, in pernicious



GLYCOLYTIC RATE IN VITRO OF NORMAL AND PERNICIOUS ANEMIA BLOOD

NG = NORMAL OF SCHMITZ-GLOVER -----17 CASES
 NL = NORMAL OF FALCON-LESSES -----8 CASES
 AE = PERNICIOUS ANEMIA BLOOD BEFORE TREATMENT-9 CASES
 AR = PERNICIOUS ANEMIA BLOOD AT HEIGHT OF RETICULOCYTE RESPONSE-9 CASES

CHART I

anemia in early remission the glycolytic activity is more rapid than in normal blood. Per unit number of red blood cells (with all factors constant), the only difference between the two is the increased number of reticulocytes. This one factor probably accounts for the accelerated rate of glycolysis which is present in the blood in early remission. As suggested by Barer, Needles and Baldrige (5), this increase in the glycolytic activity may indicate that immature red blood cells have a more active metabolism than adult red blood cells.

Five of the patients had higher red cell counts a week after therapy

was instituted, two the same, and one less, yet in all instances the rate of glycolysis was faster with the presence of increased numbers of reticulocytes. In Case 6, the rate of glycolysis was determined before therapy, at the height of the reticulocyte response, and after the immature red cells returned to normal numbers. The glycolytic activity was accelerated when the reticulocytes were present in increased numbers, although the red cell count in this instance was the lowest. This is added evidence that the type of cell present is as important as the number of cells.

According to Glover, Daland, and Schmitz (6), there is a difference of 0.004 mgm per hour in the rate of glycolysis with white cell counts showing a variation of 10,000 per cu mm. In the 9 cases of pernicious anemia, the greatest difference in white cells was 6000 per cu mm. It would seem that the effect of the white blood cells on the rate of glycolysis in pernicious anemia is extremely small, the glycolytic activity being primarily correlated with the number and type of red cells which were present.

CONCLUSIONS

1 The average rate of glycolysis per hour in vitro in the blood of 9 cases of pernicious anemia in relapse was 6.4 mgm, the range being 5 to 9 mgm at 37° C.

2 The average rate of glycolysis per hour in the blood of 9 cases of pernicious anemia in early remission was 10.2 mgm, the range being 8 to 13 mgm at 37° C.

3 The average rate of glycolysis per hour per million red blood cells in blood of normal individuals is 3.5 mgm, in pernicious anemia in relapse 3.7 mgm, in pernicious anemia in early remission 5.4 mgm at 37° C.

4 The retarded rate of glycolysis in pernicious anemia is proportional to the red cell decrease.

5 With all other factors constant, the increase in the rate of glycolysis per hour per million red cells in pernicious anemia in early remission is associated with the increased number of reticulocytes present.

BIBLIOGRAPHY

- 1 Maclean, H., and Weir, H. B., *Biochem J*, 1915, ix, 412. The Part Played by the Different Blood Elements in Glycolysis.
- 2 Folin, O., and Wu, H., *J Biol Chem*, 1920, xli, 367. A System of Blood Analysis Supplement I. A Simplified and Improved Method for Determination of Sugar.
- 3 Falcon-Lesses, M., *Arch Int Med*, 1927, xxxix, 412. Glycolysis in Normal and in Leukemic Blood.
- 4 Schmitz, H. L., and Glover, E. C., *J Biol Chem*, 1927, lxxiv, 761. Glycolysis in Leukemic Blood.
- 5 Barer, A. P., Needles, R. J., and Baldrige, C. W., *Proc Soc Exper Biol and Med*, 1929, xxvii, 176. A Study of the Metabolism of Reticulocytes.
- 6 Glover, E. C., Daland, G. A., and Schmitz, H. L., *Arch Int Med*, 1930, xlv, 46. The Metabolism of Normal and Leukemic Leukocytes.

PLASMA PROTEIN CHANGES AND SUSPENSION STABILITY OF THE BLOOD IN LOBAR PNEUMONIA

By JOHANNES K MOEN AND HOBART A REIMANN

(From the Department of Medicine University Hospital, University of Minnesota Medical School Minneapolis)

(Received for publication November 12 1932)

Numerous studies have been made on the relation of fractional blood protein changes to bacterial infection and immunity. The subject is of especial importance in view of the renewed interest regarding the association of immune bodies with the globulins and recent studies in regard to the effects of blood protein changes and plasma viscosity on specific and non specific agglutination reactions (1, 2). Since the early experiments of Langstein and Mayer (3), it has been generally found that a relative or absolute increase of the globulin fraction and a decrease in the albumin fraction occur during and after various bacterial infections. Glaessner (4) and later Hurwitz and his co workers (5) showed that increases in globulin may occur during the course of immunization as well. They believed that the globulin increase indicated disturbed metabolism and that no direct parallelism existed between the amount of globulin and the degree of immunity. There was no constant relationship between the increased amount of globulin and the antitoxic potency of prepared sera. Similar findings were noted by Schmidt and Tuljtschinskaja (6) and others. Studies on fractional blood protein changes in malaria, typhoid fever and after typhoid vaccination were made by Lloyd and Paul (7). They observed a marked decrease in the albumin fraction, an increase in the euglobulin and slight or no changes in the total globulin during the acute stage of typhoid fever and malaria. Similar but less marked changes followed vaccination with typhoid bacilli.

Many studies have been made on the blood protein changes in lobar pneumonia. Kumpf (8) collected data from 29 reports. It was generally agreed that the total protein was lowered, the fibrinogen and globulin increased and the albumin decreased in amount. No particular attention, however, was given to the speed with which changes in plasma proteins occur after the onset of infection or the time required before the normal equilibrium was regained.

Increase of plasma globulin and fibrinogen during infections causes a decrease of the suspension stability of the blood and consequent reduction of the sedimentation time of erythrocytes. The correlation between plasma protein changes and sedimentation time has been demonstrated

repeatedly (9, 10, 11, 12, 13) In a statistical analysis of the effect of blood protein fractions on the sedimentation rates in a large series of mixed clinical cases, Westergren and his co-workers (12) found a distinct correlation for fibrinogen and globulin and a negative correlation for albumin Other factors, including hydremia, anemia, changes in cell volume and color index, also influence the suspension stability (14, 15, 16, 17) Possibly because of these other factors, a number of observers have been unable to establish a constant correlation between fractional protein changes and sedimentation time (18, 19, 20, 21) In two reports (19, 21) the changes in the amount of fibrinogen, which is known to play the most important role in suspension stability, were not measured

The studies reported in this paper were undertaken to determine the correlation between fractional plasma protein changes, plasma viscosity and the sedimentation time of erythrocytes during and after lobar pneumonia

METHODS

Plasma Protein Determinations (Supervised by Dr Grace Medes) Twenty cc of blood was withdrawn from the arm vein with minimum stasis, and oxalated Plasma protein fractions were determined by Berglund's modification (22) of Howe's method using sodium sulphate as a salting-out agent Nitrogen determinations were made by the micro-Kjeldahl technic The average normal protein values obtained with this method in grams per 100 cc plasma were

Total protein	6.67		
Albumin	4.07		
		{	fibrinogen 29
			euglobulin 68
Globulin	2.59	{	pseudoglobulin I 94
			pseudoglobulin II 68

Sedimentation time At the risk of confusing comparative data, the sedimentation time was observed by employing the usual standard hospital apparatus in place of special tubes Nine-tenths cc of blood was drawn into a tuberculin syringe (4 mm diameter) containing 0.1 cc of a 10 per cent solution of sodium citrate The syringe was inverted a number of times to insure thorough mixing, the needle was removed and replaced by a short piece of rubber tubing The end of the tube was bent back along the barrel of the syringe and fastened with a rubber band The syringe was placed in a vertical position, stoppered and observed at room temperature The number of minutes required for the erythrocyte level to reach the 0.6 cc mark was arbitrarily considered to be the sedimentation time The average normal sedimentation time by this method was 3 hours or more

The Hess viscosimeter was used for the determination of plasma viscosity According to the technic employed the viscosity of normal plasma was from 1.6 to 1.7

- xxiii, 325 Plasma Protein, Red Cell Sedimentation and Serum Lability of the Blood in Tuberculosis
- 21 Oelkers, H A, and Ohnesorge, G, Ztschr f d ges exper Med, 1931, lxxviii, 156 Eiweissgehalt und kolloidosmotischer Druck im Serum von Fieberkranken
- 22 Berglund, H, Scriver, W, and Medes, G, In Press Plasma and Urinary Proteins Lea and Febiger, Philadelphia, Pa
- 23 Soule, H C, Buckman, T E, and Darrow, D C, J Clin Invest, 1928, v, 229 Blood Volume in Fever
- 24 Buccianti, E, Il Policlinico, Sez Med, 1931, xxxviii, 138 Il Comportamento del Volume Totale di Plasma Circolante nelle Malattie Infettive Acute
- 25 Loeper, M, Ravier, J de Sèze, and Lebert, Compt rend Soc de biol, 1929, ci, 423 La Globulinémie à la Période Critique de la Pneumonie
- 26 Lohr, H, Ztschr f d ges exper Med, 1922, xxvii, 1 Die Beeinflussung der Blutkörperchensenkungsgeschwindigkeit durch Reizstoffe
- 27 Reimann, H A, Medes, G, and Fisher, L C To be published

THE BLOOD IN CASES OF UNEXPLAINED GASTRIC ANACIDITY¹

By W SCOTT POLLAND²

(From the Department of Medicine, Stanford University Medical School, San Francisco)

(Received for publication January 23 1933)

The question of the relationship of gastric anacidity to anemia has recently been the subject of renewed interest and study Some writers believe that people with unexplained achlorhydria are potential cases of pernicious anemia, subacute combined sclerosis or hypochromic anemia³ (microcytic, idiopathic, achylic, simple) Others, however, feel that the view that anacidity bears a causal relationship to these disorders requires further support and that in most cases defective gastric secretion is a harmless variation from the normal

While it must be admitted that many patients with anemia have a gastric anacidity no comparative study, as far as we know, has been made of the blood in comparable groups of people on the one hand with defective and on the other with normal gastric juice, inasmuch as a control of this sort seems fundamental in interpreting the problem we have carried out such observations and they are herewith reported

LITERATURE

Einhorn (1) (1892) demonstrated that anacidity can be present for several years without the development of pernicious anemia Later (1903) he (2) found some degree of anemia in twelve out of fifteen cases and in four the hemoglobin was below 60 per cent Three of these patients probably had pernicious anemia

At the International Congress of Medicine in London (1913) Faber (3) proposed that the anemia frequently associated with achylia gastrica, was a secondary phenomenon produced by an absence of gastric secretion Among two hundred and one cases of achylia gastrica, Faber found fifty-nine with a hemoglobin below 80 per cent Twenty two were examples of Addison's anemia, and thirty seven, of simple anemia Twenty two of the cases of simple anemia were severe, the hemoglobin being below 65 per cent Weinberg (4) (1920) states that the blood picture is often

¹ Supported in part, by a grant from the Rockefeller Fluid Research Fund of the School of Medicine Stanford University

² Fellow in Medicine, National Research Council, 1932-1933

³ For the purpose of convenience, this condition will be referred to in this paper as hypochromic anemia

abnormal in anacidity, but Alsberg (5) (1921) noted anemia in only one of his seventy cases. Hurst has maintained for many years that the absence of hydrochloric acid from the gastric secretion is the essential predisposing cause of Addison's anemia and subacute combined sclerosis. For his conceptions, the reader is referred to his papers which have been collected into a single volume (6).

Hunter (7) (1923) analyzed the findings in sixty patients with anacidity most of whom had chronic diarrhea. Seven had a moderate or severe secondary anemia. Faber and Gram (8) (1924) restudied the problem in a new series of ninety cases excluding those with carcinoma, pernicious anemia or tuberculosis. Anemia was defined as a hemoglobin value less than 96 per cent in men and 88 per cent in women. The percentage of anemia was 41. Schneider and Carey (9) (1928) found in fifty-one cases of achlorhydria, seven with a high color index and seven with a secondary anemia. Borgbjaerg and Lottrup (10) (1929) state that 41 per cent of one hundred and thirty-four cases had a secondary anemia, usually of a mild degree, and that several had a color index over one. Lerman, Pierce and Brogan (11) (1932) observed in normal individuals that the red cell count and hemoglobin tended to vary directly with the level of gastric acidity.

Many cases have recently been reported, usually under the heading of hypochromic anemia. This disorder is described as an anemia with small erythrocytes and a low color index, occurring in middle aged women who have an anacidity.

A number of writers (Witts (12), Waugh (13), McCann and Dye (14), Dameshek (15), Mills (16), Davies (17), and VanderHoof and Davis (18)) believe that this syndrome is a specific disease and that the anacidity is an important etiological factor. However, Bloomfield (19) has critically reviewed the subject and pointed out that various degrees of anemia may be associated with anacidity and that all types of gastric secretion may be associated with anemia.

The fact that anacidity may precede pernicious anemia by an interval of from 3 months to 25 or more years, has been reported by at least eighteen authors. Ivy, Morgan and Farrell (20) have been able to collect a total of thirty-six reported cases. In a review of the hereditary aspects of achlorhydria in pernicious anemia, Conner (21) cites thirty-seven authors. He reports that among one hundred and fifty-four relatives of one hundred and nine patients having pernicious anemia, the percentage of anacidity was 25.9, whereas among those of a control group, the percentage was 15.2.

Castle (22), (23), (24), (25) has demonstrated that normal human gastric juice can produce by interacting with beef muscle, a substance as effective as liver in promoting blood and clinical improvement in pernicious anemia. He believes that pernicious anemia is a deficiency disease.

brought about by defective digestion of protein, as a result of achylia gastrica. In support of this hypothesis, he reports a patient without anemia and three with hypochromic anemia, all of whom had an anacidity to histamine, but who nevertheless had the necessary hematopoietic stimulating substance present in their gastric juice. Barnett (26) using the same method of biologic assay was unable to confirm his results on two cases without anemia known to have had an anacidity for several years.

MATERIAL AND METHODS

The material consisted of twenty six males and twenty-two females with unexplained anacidity as demonstrated by the previously described histamine technique (27). The anacidity was usually an accidental finding and most of the patients did not have a primary digestive complaint. Patients with carcinoma, pernicious anemia, hyperthyroidism, syphilis, fever, bleeding, or chronic wasting diseases, such as tuberculosis, were excluded. The known duration of the anacidity to histamine varied from 1 month to 4 years and 8 months. The average for the males was 27 months, and for the females, 19.4 months. In practically all of the cases, the family history was negative or no data was obtainable. In one case it was reported that a brother had died of pernicious anemia.

The following blood examinations were made: red and white cell counts, including a differential, hemoglobin (Sahli) and color index. In order to obtain more detailed data on the condition of the blood fifteen males and ten females were examined by workers specially trained in hematological methods. Red and white cell counts were made with standardized counting chambers and pipettes. In the differential counts of the leukocytes 200 cells were enumerated. Hemoglobin was determined by the oxygen capacity method of Van Slyke and Neill (28) and color indices were calculated on the basis of a correspondence of 5,000,000 corpuscles and 91 per cent hemoglobin equal to 15.6 grams of hemoglobin per 100 cc of blood. Platelets and reticulocytes were counted by a method devised by Dr. Harry A. Wyckoff of the Stanford Medical School. The average diameter of the red corpuscles was measured by the Price Jones technique (29). The frequency distribution of the corpuscular diameters was determined with an ocular micrometer, measuring 200 corpuscles in freshly dried smears, fixed and stained with Wright's stain. Icterus indices and Van den Bergh tests were done in the usual manner.

For a control group, a similar study was made of fifteen males and eleven females of about the same age and physical status, who had, however, free acid in their gastric secretion. These subjects were chosen seriatim from a miscellaneous group of hospital patients, and the criteria for selection were the same as for the anacidity group.

RESULTS

In Tables 1 and 2 the age, diagnosis and test meal findings⁴ are recorded for the control group. Patients with a wide variety of disorders and with every type of gastric secretion were used.

TABLE 1

Age, diagnosis and gastric secretory findings in male controls

Case	Age	Clinical diagnosis	Volume	Free acidity	Total acidity
	<i>years</i>		<i>cc</i>	<i>m Eq/L</i>	<i>m Eq/L</i>
1	31	Indigestion, psychoneurosis	60	92	102
2	58	Abdominal pain (unexplained)	23	66	76
3	42	Pneumonoconiosis	21	40	56
4	42	Abdominal pain (unexplained)	23	78	96
5	48	Duodenal ulcer	42	138	142
6	57	Gastric ulcer	50	94	102
7	46	Psychoneurosis	50	65	66
8	32	Psychoneurosis	20	66	78
9	46	Irritable colon	29	100	113
10	28	Psychoneurosis	27	124	130
11	40	Indigestion	17	112	120
12	56	Duodenal ulcer	35	84	99
13	55	Gastric ulcer	36	98	106
14	68	Chronic hepatitis ?	2	25	35
15	46	Indigestion	35	50	58
Average	46.3		31.6	82	92

TABLE 2

Age, diagnosis and gastric secretory findings in female controls

Case	Age	Clinical diagnosis	Volume	Free acidity	Total acidity
	<i>years</i>		<i>cc</i>	<i>m Eq/L</i>	<i>m Eq/L</i>
1	58	Arteriosclerosis and hypertension	42	105	113
2	63	Arteriosclerosis and hypertension	2	25	35
3	36	Duodenal ulcer	55	52	62
4	71	Psychoneurosis	35	91	96
5	41	Psychoneurosis	18	91	101
6	48	Hypertension	2	6	20
7	61	Auricular fibrillation	12	60	70
8	76	Cholelithiasis, diabetes mellitus	16	86	100
9	56	Chronic cholecystitis	52	74	87
10	57	Diabetes mellitus	32	23	43
11	63	Arteriosclerosis, diabetes mellitus	40	104	108
Average	57.2		27.8	65.2	76.8

⁴ The highest 10 minute secretory volumes and highest free and total acidity after histamine are alone recorded.

In Tables 3, 4, 5 and 6, a detailed report of the blood of the subjects who were especially studied is presented and in Table 7 the findings are summarized. A number of points revealed by those tables will be discussed.

Hemoglobin The hemoglobin in the male anacidity patients ranged between 65.9 and 102 with an average of 87.6 per cent, in the controls between 80.2 and 100.9 with an average of 93.3 per cent. The average grams per cent for the anacidity group was 14.9, and for the control group, 16.07.

Hemoglobin in the female anacidity patients ranged between 54 and 95 with an average of 83.9 per cent, in the controls the values lay between 52.2 and 97.9, with an average of 85.8 per cent. The average grams per cent for the anacidities was 14.42, and for the controls 14.68. The following cases had a marked lowering of hemoglobin: male anacidity Case 8, female anacidity Case 11, and female control Case 11. Otherwise, the variations were about the same in both series.

Red cell counts There was no striking difference in the red cell counts of the two groups. Male anacidity Cases 1 and 8, female anacidity Case 9, and female control Case 11 had counts definitely below normal.

Color index The majority of the color indices were about .9 or higher. Cases 2, 8 and 11 of the female anacidities, and Case 11 of the female controls, had indices below .8. More controls than anacidities had indices above 1.00.

White cell count All of the counts were within the range usually accepted for normal people. No anacidity case had a definite leukopenia nor was the differential count unusual in any of the cases.

Platelets These varied considerably in both series, but were considered to be within the range of normal.

Reticulocytes Cases 4, 5 and 11 of the female anacidity group, and Case 6 of the male control group, showed a slight reticulocytosis.

Icterus index and van den Bergh A number of individuals among both the "anacidities" and the controls had either an icterus index or Van den Bergh slightly above normal.

Price-Jones curve There was no significant difference as regards the variation in size of red cells between the anacidities or controls.

Smear Reports such as normal smear, slight anisocytosis, moderate polychromasia, etcetera, were not uncommon in either series. The impression of the worker studying the smear did not always correspond to the type of Price-Jones curve recorded, but usually there was a close agreement between the two. Case 11 of the female anacidities and Case 11 of the female controls had definitely abnormal smears.

TABLE 3
Blood studies in male control cases

Case*	Hemo- globin	Red blood cells	Color index	White blood cells	Differential			Plate lets	Reticu- locytes	Van den Bergh		Ic- terus index	Price Jones				Smear
					Polys	Lympha	Monos			Direct	Indi- rect		Lower size	Upper size	Apex	Aver- age	
	per cent	10 ³		10 ³	per cent	per cent	per cent	10 ³	per cent				mi- crons	mi- crons	mi- crons	mi- crons	
1	16.5	5.13	0.93	13.4	56	38	5	359	2.0	—	0.8	9.0	5.62	8.75	7.50	6.75	Slight anisocytosis
2	16.8	4.92	0.99	9.5	70	24	4	510	0.0	—	0.3	6.0	5.62	10.00	7.50	7.40	Normal
3	17.1	5.00	0.99	7.2	54	40	3	340	0.0	—	0.2	5.8	6.25	10.00	7.50	7.77	Normal
4	15.7	4.14	1.10	10.9	57	38	4	368	1.7	—	0.5	7.0	5.62	9.37	7.50	7.63	Slight anisocytosis
5	16.2	4.85	0.98	13.4	59	38	3	528	0.1	—	1.8	15.0	6.25	9.37	7.50	7.73	Normal
6	13.8	4.24	0.95	8.8	63	30	5	487	2.8	—	—	—	5.62	9.37	7.50	7.35	Normal
7	14.6	4.90	0.86	9.3	67	22	7	461	0.0	—	0.4	7.5	5.00	8.75	6.87	7.17	Slight anisocytosis
8	14.6	4.35	0.98	4.9	36	55	5	275	0.1	—	0.3	6.3	6.25	8.75	7.50	7.38	Normal
9	16.3	4.67	1.03	10.7	49	46	4	317	0.1	—	0.5	7.6	5.62	9.37	7.50	7.57	Normal
10	17.2	5.04	1.00	9.8	52	35	7	297	0.0	—	0.5	6.0	5.62	9.37	7.50	7.40	Moderate polychromasia
11	15.7	5.05	0.91	6.1	49	45	2	273	0.0	—	0.8	8.1	5.62	8.75	6.87	7.60	Slight microcytosis
12	15.8	4.75	0.97	12.7	56	36	3	502	0.0	—	0.2	6.6	5.00	10.00	8.12	7.77	Normal
13	17.3	5.47	0.93	9.9	51	27	5	443	0.0	—	1.0	8.0	5.00	9.37	6.87	7.19	Normal
14	16.1	4.45	1.07	8.2	51	39	5	134	1.1	—	4.5	15.7	5.00	9.37	8.12	8.06	Moderate anisocytosis
15	17.5	5.00	1.00	8.2	53	41	6	149	0.2	—	0.3	9.7	5.62	9.37	7.50	7.46	Slight anisocytosis
Average	16.1	4.79	0.98	9.5	56	37	5	363	0.54	—	0.8	8.5	5.58	9.33	7.46	7.48	

* Numbers correspond to those of Table 1

TABLE 4
Blood studies in female control cases

Case*	Hemo globin	Red blood cells	Color index	White blood cells	Differential			Plate lets	Reticu- locytes	Van den Bergh		Ic- terus index	Price Jones				Smear
					Poly*	Lympha*	Monos			Direct	Indi- rect		Lower size	Upper size	Apex	Aver- age	
	grams per cent	10 ⁶		10 ³	per cent	per cent	per cent	10 ³	per cent				ms crons	mi crons	mi crons	mi crons	
1	13.8	4.47	0.91	9.8	68	24	3	345	0.0	—	0.5	6.6	5.62	9.37	7.50	7.36	Normal
2	12.6	4.37	0.88	12.6	70	24	4	351	0.1	—	0.3	5.8	5.62	9.37	7.50	7.40	Normal
3	15.3	5.09	0.87	9.2	60	32	5			—	0.8	8.9	4.35	9.37	6.25	6.73	Definite microcytosis
4	16.1	4.40	1.06	9.9	61	26	7	431	1.0	—	1.0	9.5	6.25	9.37	8.12	7.94	Normal
5	16.3	4.75	1.00	9.1	62	32	3	346	0.8	—	0.5	7.0	6.25	9.37	7.50	7.53	Moderate anisocytosis
6	15.1	4.20	1.06	11.3	70	25	2	335	1.4	+	1.0	5.7	5.62	9.37	6.87	7.40	Normal
7	15.1	4.04	1.09	9.2	55	29	7	327	0.1	—	0.3	7.8	5.62	11.25	8.12	7.72	Normal
8	15.7	4.65	0.99	14.0	56	32	6	260	0.0	—	0.2	6.9	5.62	9.37	7.50	7.62	Normal
9	15.8	4.49	1.00	8.0	69	15	13	242	0.9	—	0.5	6.5	5.62	10.00	8.12	7.73	Slight anisocytosis
10	16.8	4.22	1.15	7.5	57	40	3	236	1.0	—	0.3	6.5	5.62	8.75	8.12	7.34	Slight anisocytosis
11	8.9	3.68	0.7	8.2	61	33	3	379	0.4	—	0.3	5.0	5.00	8.75	6.25	6.51	Extensive anisocytosis
Average	14.7	4.40	0.98	9.9	63	28	5	296	0.5	—	0.5	6.3	5.56	9.48	7.44	7.39	

* Numbers correspond to those of Table 2

TABLE 5
Age, diagnosis and blood studies in male anacidity cases

Case	Age	Clinical diagnosis	Hemo globin	Red blood cells	White blood cells	Differential		Plate- lets	Ratio sub- cytes	Van den Bergh		Ic- terus Index	Price-Jones				Smear
	years		grams per cent	10 ⁶	10 ³	Polys	Lymphs.	Monos.		Dy rect	Indi- rect		Lower size	Upper size	Apex	Aver age	
						per cent	per cent	per cent	10 ³	per cent			mi- crons	mi- crons	mi- crons	mi- crons	
1	73	Arteriosclerosis, myocarditis	12.2	3.54	10.6	80	11	0	574	0.3	—	11.2	5.62	8.75	7.50	7.44	Normal
2	50	Indigestion	15.8	4.92	13.0	75	18	5	221	0.0	0.8	5.6	5.62	9.37	7.50	7.81	Normal
3	69	Adenoma of thyroid	15.7	4.64	9.8	69	23	5	269	0.6	0.3	5.6	5.00	9.37	7.50	7.28	Slight anisocytosis
4	46	Irritable colon acute rosacea	15.5	4.60	8.8	73	20	1	349	0.4	0.3	10.2	5.62	9.37	6.87	7.24	Normal
5	47	Indigestion	16.5	5.10	9.9	71	63	8	183	0.0	0.8	8.2	5.62	10.00	6.87	7.40	Normal
6	48	Epilepsy	17.6	5.24	0.97	63	27	9	387	0.6	0.2	11.0	5.00	10.00	8.12	7.64	Slight macrocytosis
7	44	Chronic arthritis	14.8	4.33	1.00	70	27	2	473	0.2	0.2	6.1	5.00	8.75	6.87	7.43	Slight poikilocytosis
8	61	Arteriosclerosis	11.3	3.40	1.99	65	21	9	686	0.1	0.6	7.6	6.26	9.37	7.50	7.54	Slight anisocytosis
9	60	Arteriosclerosis hypertension	12.3	4.12	0.95	55	32	3	151	0.2	0.2	4.8	5.62	10.00	8.12	8.31	Slight anisocytosis
10	67	Arteriosclerosis chronic prostatitis	14.0	4.42	0.93	73	18	6	296	0.6	1.0	7.8	6.25	9.37	7.50	7.32	Slight anisocytosis
11	58	Cerebral arteriosclerosis	17.5	4.31	1.03	95	01	10	490	0.8	0.6	9.0	5.62	9.37	7.50	7.58	Definite anisocytosis
12	44	Indigestion	15.7	4.54	1.02	85	07	23	345	0.3	0.5	7.6	5.62	9.37	7.50	7.65	Normal
13	62	Angina pectoris	16.0	4.33	1.01	69	64	28	338	0.0	0.8	9.0	5.62	9.37	7.50	7.40	Slight anisocytosis
14	75	Arteriosclerosis myocarditis	13.4	4.62	0.85	58	30	5	410	0.6	0.2	5.6	5.00	10.00	7.50	7.54	Slight anisocytosis
15	64	Chronic constipation	17.0	5.65	0.90	55	35	5	242	0.2	0.5	7.6	5.00	8.75	6.87	6.92	Normal
Average			14.9	4.55	0.97	65	24	7	361	0.5	0.5	7.4	5.49	9.48	7.41	7.40	

TABLE 6
Age diagnosis and blood studies in female anacidity cases

Case	Age	Clinical diagnosis	Hemo- globin	Red blood cells	Color index	White blood cells	Differential			Plate- lets	Retic- ulo- cytes	Van den Berg		Fe- terus index	Pucc-Jones			Smear	
							Poly.	Lympha.	Monos.			Di rect	Indl rect		Lower size	Upper size	Aver- age		
	years		grams per cent	10 ⁶		10 ³	per cent	per cent	per cent	10 ⁹	per cent			mi crons	mi crons	mi crons	mi crons		
1	51	Irritable colon	16.3	5.61	0.84	10.6	72	25	3	983	0.5	—	0.5	5.0	5.62	8.75	6.87	6.50	Slight microcytosis
2	30	No disease	18.0	6.27	0.73	7.9	60	35	2	315	0.1	—	0.5	6.8	5.6	10.62	6.87	7.23	Normal
3	47	Psychoneurosis	14.6	4.70	0.91	8.0	47	47	4	25	1.6	—	0.6	8.8	8.00	8.75	6.87	6.88	Normal
4	56	Irritable colon	14.5	4.61	0.91	6.5	68	22	6	194	4.8	—	0.7	9.0	6.62	10.00	6.87	7.41	Slight anisocytosis
5	39	Obesity hypertension	15.1	4.79	0.83	12.1	65	27	6	343	3.4	—	0.2	7.3	5.62	9.37	7.50	7.38	Slight anisocytosis
6	24	Psychoneurosis	15.5	5.00	0.80	14.3	77	15	5	440	1.2	—	0	11.5	5.62	9.37	7.50	7.65	Normal
7	65	Induration	12.7	4.23	0.87	9.3	43	47	4	248	0.1	—	0.3	5.0	6.25	10.00	7.50	7.72	Normal
8	50	No disease	12.5	3.79	0.76	8.3	59	36	4	139	0.2	—	0.2	7.8	5.63	9.37	6.87	7.09	Moderate anisocytosis
9	50	No disease	14.8	5.97	1.10	8.4	67	24	7	429	0.4	—	0.5	9.0	5.00	10.00	7.00	7.53	Normal
11	49	Periarthritis nodosa	9.3	4.23	0.84	9.6	67	19	12	516	2.6	—	0.3	6.0	3.75	10.00	7.50	7.05	Marked anisocytosis
Average			14.4	4.93	0.86	9.7	63	30	5	319	1.8	—	0.6	7.3	5.37	9.02	7.19	7.26	

TABLE 7
Summary of average findings in anacidity and control cases

	Hemo- globin	Red blood cells	Color index	White blood cells	Differential			Reticu- locytes	Van den Bergh		Icterus index	Price Jones			
					Polys	Lymphs	Monos		Direct	Indirect		Lower size	Upper size	Apex	Aver- age
	grams per cent	10 ⁶		10 ³	per cent	per cent	per cent	per cent				microns	microns	microns	microns
Male anacidities	14.90	4.55	0.97	9.6	65.4	24.3	6.5	0.46	—	0.45	7.4	5.49	9.48	7.41	7.49
Male controls	16.07	4.79	0.98	9.5	55.5	36.9	4.5	0.54	—	0.78	8.5	5.58	9.33	7.46	7.48
Female anacidities	14.42	4.93	0.86	9.7	62.5	29.7	5.4	1.76	—	0.59	7.3	5.37	9.62	7.19	7.26
Female controls	14.68	4.40	0.98	9.9	62.6	28.4	5.1	0.51	—	0.50	6.3	5.56	9.48	7.44	7.39

DISCUSSION

A study of the tables shows that many people with anacidity have subnormal blood counts when comparison is made with standard "normal" or "ideal" values. The point at issue, however, is not this indisputable fact but whether the anacidity bears a causal relationship to the hematological deviations. To settle this point controls are clearly necessary and when a group of people of similar age, sex and condition, *but with normal gastric secretion* is studied by the same methods no significant difference appears. This matter of controls has not, we believe, been properly emphasized in previous work, hence the rather generally accepted idea that anacidity *per se* leads to deficiency of the blood. If, for example, Case 11 of the female control series had had an anacidity she would doubtless have been considered a typical case of "achlorhydric hypochromic anemia" by most writers. A further complication comes from the fact that many of the achlorhydric women with hypochromic anemia reported in the literature had lost blood over long periods of time from uterine hemorrhage, in the present study all patients thought to have abnormal bleeding were eliminated.

Whether or not people with anacidity as a class are specially liable to develop pernicious anemia is a debatable question. Certainly there are a few isolated instances on record in which the anacidity has preceded the anemia by many years. However, some of the individuals in this series have had an anacidity to histamine for over four years and others are known to have had an anacidity to other test meals for a longer period of time without any impairment of health. In the male anacidity series, Case 1 was told that he had no acid in his stomach in 1926, Case 8 was told the same thing in 1908, and Case 9 had an anacidity to the fractional gruel meal in 1925.

Although anacidity is practically always part of the disease picture of Addisonian anemia, yet there are many gaps in our knowledge of the role of this defect in the production of the disease. Even though the two are intimately related, it is difficult to explain why the absence of free hydrochloric acid is so common in apparently healthy people.

CONCLUSIONS

The blood picture in twenty five cases of unexplained gastric anacidity was compared with that of an otherwise similar group of people except that they had an apparently normal gastric secretion. No significant difference was noted between the two groups. No evidence is therefore forthcoming that anacidity *in itself* leads to anemia.

BIBLIOGRAPHY

- 1 Einhorn, 1892, quoted from Faber, K, *Ann Clin Med*, 1926, iv, 788 The Intestinal Origin of Pernicious Anemia
- 2 Einhorn, M, *Med R*, 1903, lxi, 321 Remarks on Achylia Gastrica and Pernicious Anemia
- 3 Faber, K, *Berl klin Wchnschr*, 1913, l, 958 Anamische Zustände bei der chronischen Achylia gastrica
- 4 Weinberg, F, *Ztschr f angew Anat u Konstit*, 1920, vi, 289 Der Blutbefund bei der konstitutionellen Achylia gastrica
- 5 Alsberg, J, *Arch f Verdauungskr*, 1921, xxix, 328 Ein Beitrag zur Ätiologie der Achlorhydrie und Anacidität
- 6 Hurst, A F, *Essays and Addresses on Digestive and Nervous Diseases and on Addison's Anemia and Asthma* Wm Heinemann, London, 1924
- 7 Hunter, C, *Canad M A J*, 1923, xiii, 38 Analysis of Sixty Cases of Gastric Anacidity Associated Mainly with Chronic Diarrhoea and Pernicious Anemia
- 8 Faber, K, and Gram, H C, *Arch Int Med*, 1924, xxxiv, 658 Relations Between Gastric Achylia and Simple and Pernicious Anemia
- 9 Schneider, J P, and Carey, J B, *J A M A*, 1928, xci, 1763 The Clinical Significance of Primary Achlorhydria
- 10 Borgbjærg, A, and Lottrup, M C, *Acta med Scandinav*, 1929, lxxvii, 539 Blutuntersuchungen bei der Achylie, speziell mit Rückblick auf die perniziöse Anämie
- 11 Lerman, J, Pierce, F D, and Brogan, A J, *J Clin Invest*, 1932, xi, 155 Gastric Acidity in Normal Individuals
- 12 Witts, L J, *Guy's Hosp Rep*, 1930, x, 253 Simple Achlorhydric Anemia
- 13 Waugh, T R, *Arch Int Med*, 1931, xlvii, 71 Hypochromic Anemia with Achlorhydria
- 14 McCann, W S, and Dye, J, *Ann Int Med*, 1931, iv, 918 Chlorotic Anemia with Achlorhydria, Splenomegaly and Small Corpuscular Diameters
- 15 Dameshek, W, *Am J M Sc*, 1931, clxxxii, 520 Primary Hypochromic Anemia (Erythro-normoblastic Anemia)
- 16 Mills, E S, *Am J M Sc*, 1931, clxxxii, 554 Idiopathic Hypochromemia
- 17 Davies, D T, *Quart J Med*, 1931, xxiv, 447 Studies on Achlorhydria and Anemia
- 18 VanderHoof, D, and Davis, D, *Am J M Sc*, 1932, clxxxiv, 29 Anemia of the Microcytic Type in Middle-Aged Women
- 19 Bloomfield, A L, *Arch Int Med*, 1932, l, 328 Relations Between Primary Hypochromic Anemia and Chlorosis
- 20 Ivy, A C, Morgan, J E, and Farrell, J I, *Surg, Gynec and Obst*, 1931, lxi, 611 The Effects of Total Gastrectomy, Experimental Achylia Gastrica in Dogs with the Occurrence of a Spontaneous Anemia and Anemia of Pregnancy
- 21 Conner, H M, *J A M A*, 1930, xciv, 606 Hereditary Aspect of Achlorhydria in Pernicious Anemia
- 22 Castle, W B, *Am J M Sc*, 1929, clxxviii, 748 Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia I The Effect of the Administration to Patients with Pernicious Anemia of the Contents of the Normal Human Stomach Recovered after the Ingestion of Beef Muscle

- 23 Castle, W B , and Townsend, W C , Am J M Sc , 1929, clxxviii, 764
Ibid 11 The Effect of the Administration to Patients with Pernicious
Anemia of Beef Muscle after Incubation with Normal Human Gastric
Juice
- 24 Castle, W B , Townsend, W C , and Heath, C W , Am J M Sc , 1930,
clxxx, 305 Ibid III The Nature of the Reaction Between Normal
Human Gastric Juice and Beef Muscle Leading to Clinical Improvement
and Increased Blood Formation Similar to the Effect of Liver Feeding
- 25 Castle, W B , Heath, C W , and Strauss, M B , Am J M Sc , 1931,
clxxxii, 741 Ibid IV A Biologic Assay of the Gastric Secretion of
Patients with Pernicious Anemia Having Free Hydrochloric Acid and
That of Patients without Anemia or with Hypochromic Anemia Having
No Free Hydrochloric Acid, and of the Role of Intestinal Impermeability
to Hematopoietic Substances in Pernicious Anemia
- 26 Barnett, C W , Am J M Sc , 1932, clxxxiv, 24 The Significance of the
Gastric Secretions in Pernicious Anemia
- 27 Bloomfield, A L , and Polland W S , J A M A , 1929, xcii, 1508 The
Diagnostic Value of Studies of Gastric Secretion
- 28 Van Slyke, D D , and Neill, J M , J Biol Chem , 1924, lxi, 523 The
Determination of Gases in Blood and Other Solutions by Vacuum Ex
traction and Manometric Measurement I
- 29 Price Jones, C , J Path and Bact , 1920 xxiii, 371 The Diurnal Varia
tion in the Sizes of Red Blood Cells

cl,

STUDIES ON THE ACTION OF DIURETICS II THE EFFECT OF SALYRGAN UPON THE WATER CONTENT OF THE PLASMA AS MEASURED BY THE REFRACTIVE INDEX

By HENRY LENZEN SCHMITZ

(From the Lasker Foundation for Medical Research and the Department of Medicine University of Chicago Chicago)

(Received for publication April 5, 1933)

There are conflicting reports in the literature concerning the effect of the mercurial diuretics upon the water content of the plasma Saxl and Heilig (1) Bohn (2 3) and Crawford and McIntosh (4) have reported that the concentration of protein in the plasma falls before the onset of novasurol diuresis Muhling (5), Nonnenbruch (6) and Bleyer (7), on the other hand, found no such evidence of plasma dilution

In a previous publication (8) evidence was presented that salyrgan diuresis is the result of decreased reabsorption of water in the renal tubules It was suggested that this decrease in reabsorption is produced by a depressant action of the drug on the tubular epithelium The occurrence of hydremia preceding the onset of diuresis would be inconsistent with this hypothesis and would point to a primary extrarenal action, occurring alone or in conjunction with a direct action on the kidney Carefully controlled studies of the effect of salyrgan upon the refractive index of the plasma have therefore, been carried out For purposes of comparison similar studies have been made with intravenously administered physiological saline solution

PROCEDURE

The experiments were performed upon unanesthetized female dogs who had gone without food for twelve hours or more In Control Experiments 1 to 7 inclusive, and in the first seven salyrgan experiments, the animals were permitted to drink water as desired until two hours before the beginning of the experiment In all other experiments the dogs were allowed no water for five hours preceding the period of observation During the experiment the dog was tied down on a table After a period of training the animals would remain in this position for two to three hours without struggling Urine was collected from the bladder directly into graduated cylinders by an indwelling catheter Blood samples were drawn from the external jugular vein without stasis Heparin (0.01 cc of a 4 per cent solution to each cc of blood) was used to prevent clotting In Control Experiments 1, 2, 3, and 4 and in salyrgan Experiments 1 and 2 the blood

samples were from 5.5 to 6.0 cc each. In all other experiments about 1.5 cc of blood were drawn at each venepuncture. Immediately after withdrawal the blood specimens were centrifuged for two minutes at 1800 r.p.m., and the refractive index determined on a drop of the plasma in an Abbe refractometer at room temperature. The temperature seldom varied as much as one degree centigrade during the course of an experiment. A rise of one degree in the temperature was found to produce a fall of approximately 0.0001 in the refractive index of the plasma. Changes in temperature of less than one degree were therefore ignored. On the few occasions when greater variations than this occurred the refractive index was corrected correspondingly.

RESULTS

(a) Control experiments

The refractive index of the plasma was found to fluctuate considerably under controlled conditions (Figs 1A and 1B). Of particular interest in connection with this investigation are the decreases which occurred in Control Experiments 3, 4, 5, 6 and 7. In these five experiments the index

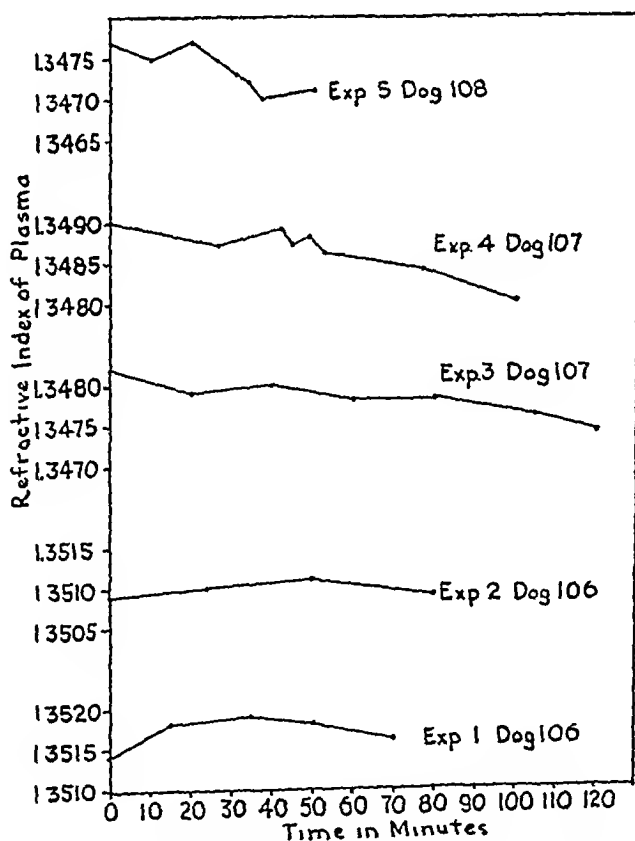


FIG 1A CONTROL EXPERIMENTS 1, 2, 3, 4 AND 5

In Experiments 1, 2, 3, 5 and 10 the index did not vary more than plus or minus 0.0002 from the control period level. In Experiment 9 a temporary increase of 0.0005 occurred after the administration of salyrgan. The index then returned to and remained at approximately the control period level until diuresis began. In another instance (Experiment 8) a more persistent rise in the index occurred. In the remaining three experiments

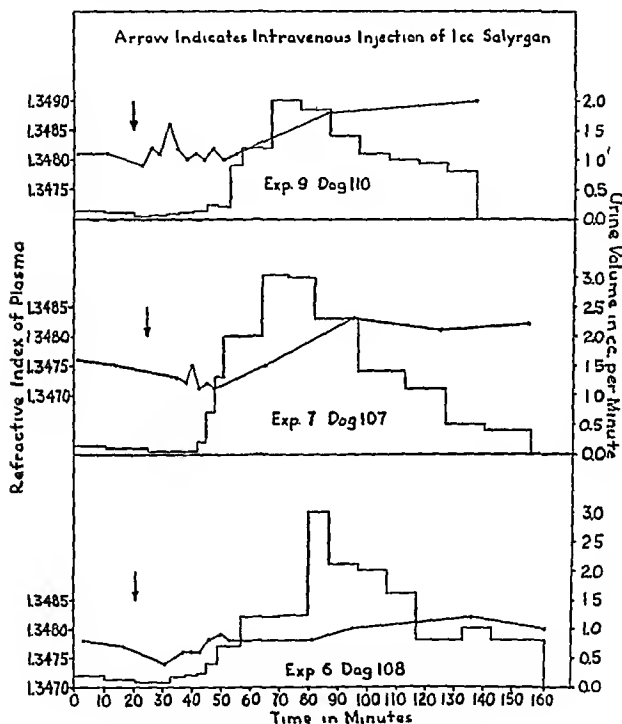


FIG 2B SALYRGAN EXPERIMENTS 6 7 AND 9

(4, 6 and 7) the index fell 0.0006, 0.0003 and 0.0004 (0.3, 0.15 and 0.2 gram per cent of protein) respectively. Decreases of equal magnitude and occurring within a similar period of time were observed in the control experiments without any subsequent increase in urine volume.

In all ten salyrgan experiments the refractive index of the plasma increased gradually during the early part of diuresis. When the index had

a considerable rise occurred. These results may, however, be merely a matter of chance. A rise in the index occurred in Experiment 1, and the index remained fairly constant in Experiment 2, although water was withheld for only two hours in both of these experiments. In the control period of salyrgan Experiment 10, on the other hand, a precipitous drop in the index occurred even though the dog had received no water for five hours.

The urine output in all of the control experiments either remained at about the same level or slowly declined during the period of observation. The spontaneous decreases in the index of refraction of the plasma were never followed by diuresis.

(b) Salyrgan experiments

No consistent change in the refractive index of the plasma was observed before the onset of salyrgan diuresis (see Figures 2A, 2B, and 2C)

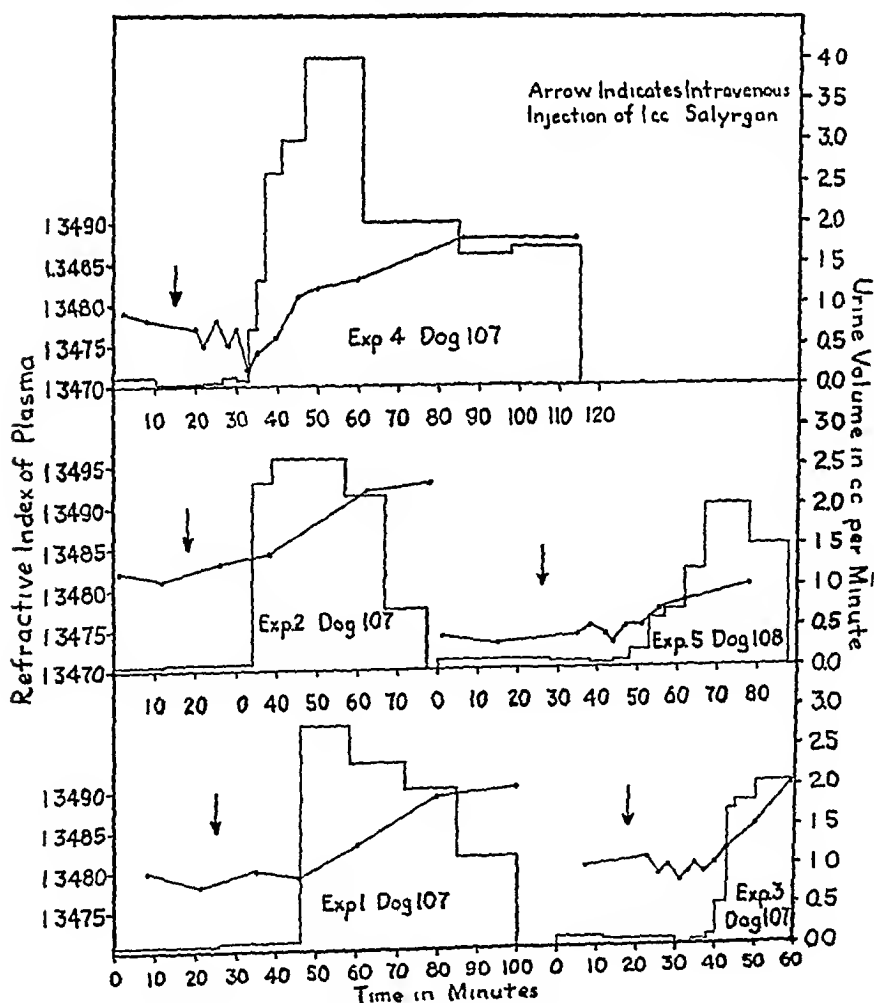


FIG 2A SALYRGAN EXPERIMENTS 1, 2, 3, 4 AND 5

a considerable rise occurred. These results may, however, be merely a matter of chance. A rise in the index occurred in Experiment 1, and the index remained fairly constant in Experiment 2, although water was withheld for only two hours in both of these experiments. In the control period of salyrgan Experiment 10, on the other hand, a precipitous drop in the index occurred even though the dog had received no water for five hours.

The urine output in all of the control experiments either remained at about the same level or slowly declined during the period of observation. The spontaneous decreases in the index of refraction of the plasma were never followed by diuresis.

(b) Salyrgan experiments

No consistent change in the refractive index of the plasma was observed before the onset of salyrgan diuresis (see Figures 2A, 2B, and 2C)

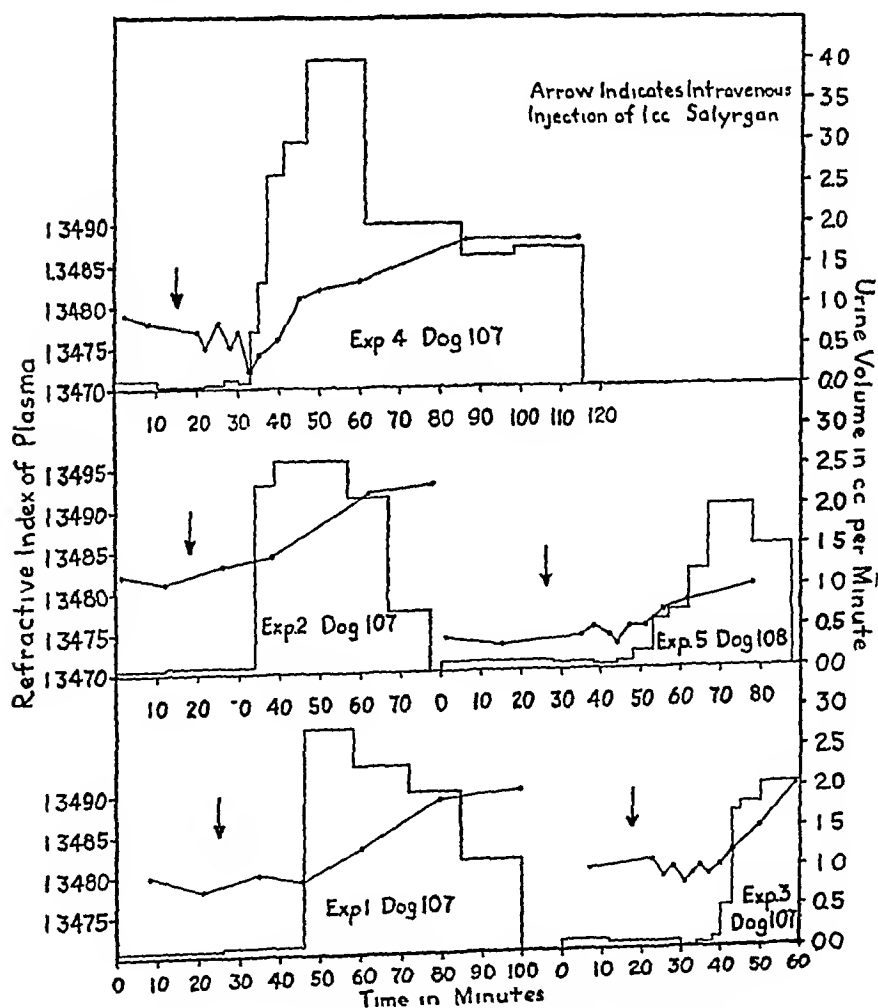


FIG 2A SALYRGAN EXPERIMENTS 1, 2, 3, 4 AND 5

In Experiments 1, 2, 3, 5 and 10 the index did not vary more than plus or minus 0.0002 from the control period level. In Experiment 9 a temporary increase of 0.0005 occurred after the administration of salyrgan. The index then returned to and remained at approximately the control period level until diuresis began. In another instance (Experiment 8) a more persistent rise in the index occurred. In the remaining three experiments

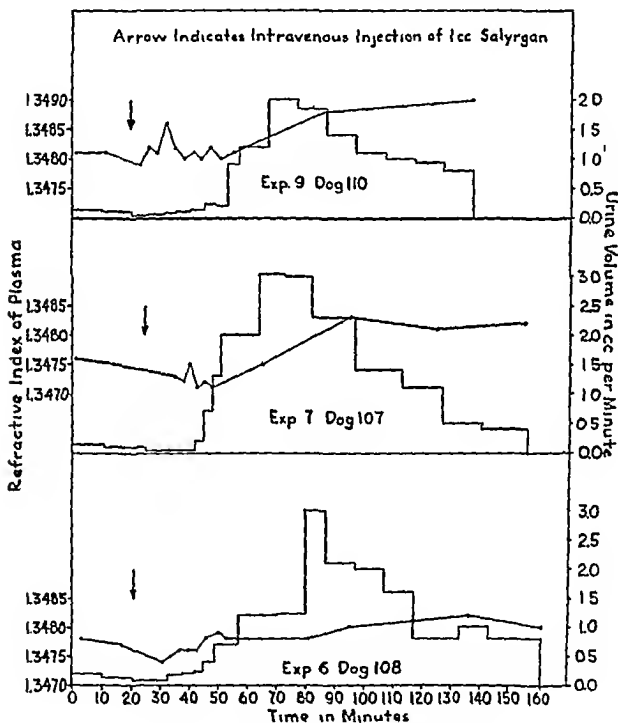


FIG 2B SALYRGAN EXPERIMENTS 6, 7 AND 9

(4, 6 and 7) the index fell 0.0006, 0.0003 and 0.0004 (0.3, 0.15 and 0.2 gram per cent of protein) respectively. Decreases of equal magnitude and occurring within a similar period of time were observed in the control experiments without any subsequent increase in urine volume.

In all ten salyrgan experiments the refractive index of the plasma increased gradually during the early part of diuresis. When the index had

risen approximately 0.0010 (representing an increase of 0.5 gram per cent in the protein concentration) no further increase occurred

(c) *Saline experiments*

Varying amounts of 0.9 per cent saline solution were injected intravenously at the rate of 10 cc per minute (see Figures 3A and 3B). In

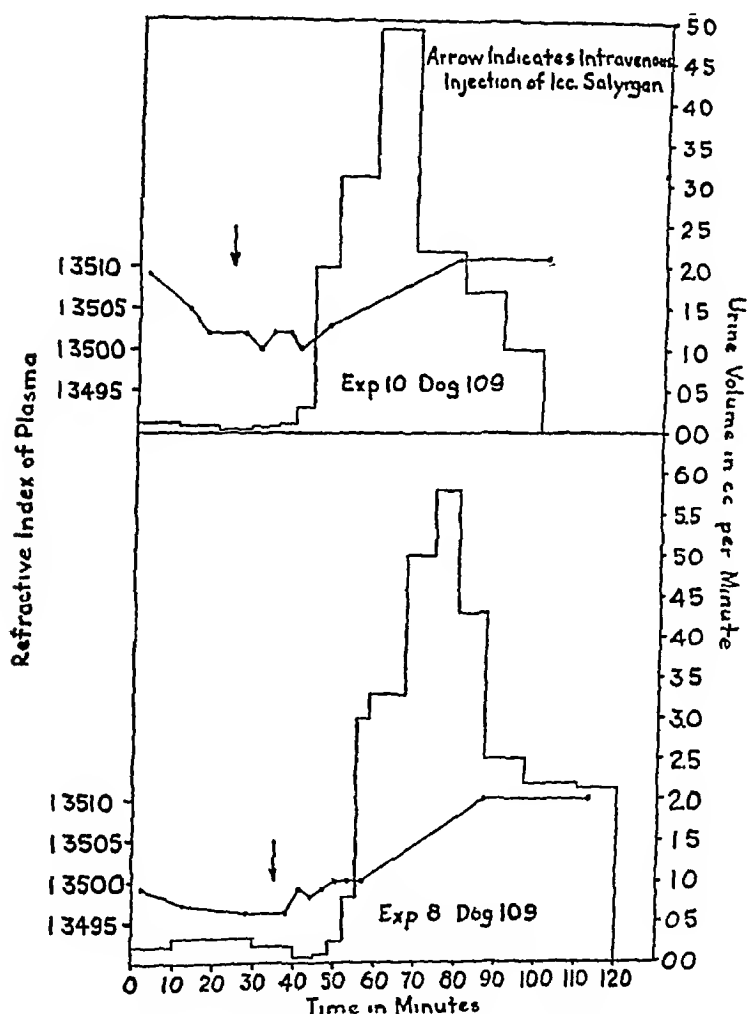


FIG 2C SALYRGAN EXPERIMENTS 8 AND 10

Experiments 1 and 2 the index of refraction of the plasma fell 0.0006 and 0.0009, respectively, without any increase in the urine volume. In the other four experiments, in which the index was lowered more than 0.0010, diuresis occurred. There was no correlation, however, between the magnitude of the urine volume and the degree to which the index of refraction

was decreased. Only slight diuresis occurred in Experiments 3 and 5 in which the index fell 0.0015 and 0.0023 respectively, whereas the urine output was augmented considerably in Experiments 4 and 6, in which the index dropped 0.0017 and 0.0013, respectively below the control period level.

COMMENT

Bohn has suggested that the contradictory reports in the literature concerning the effect of novasurol upon the water content of the plasma are

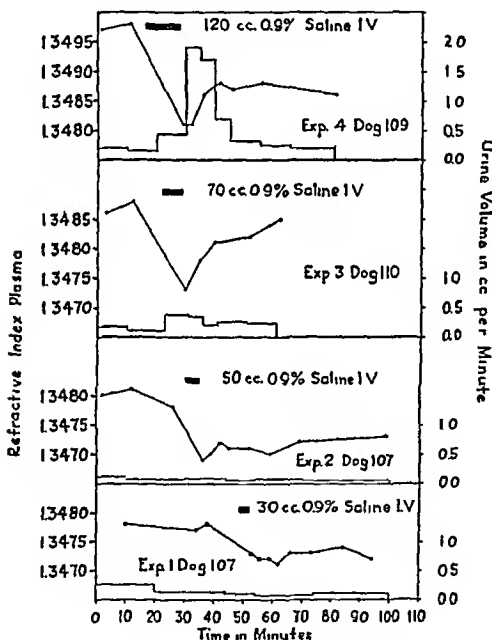


FIG 3A SALINE EXPERIMENTS 1, 2, 3 AND 4

due to the fact that blood samples were taken at different times and that the temporary hydremia was missed by some investigators. In the present experiments samples of blood were taken at approximately three minute intervals during the period between the administration of salyrgan and the onset of diuresis. There is no evidence in these experiments that diuresis is preceded by a mobilization of fluid from the tissue spaces. The refractive index of the plasma showed no consistent change before the onset

of diuresis and such decreases as were observed fell within the limits of variation noted in the control experiments

The argument has been advanced that the kidneys respond so promptly with diuresis when a shift of fluid from the tissues to the blood occurs that it is impossible to demonstrate appreciable dilution of the plasma. The results of the saline experiments do not support such a hypothesis. They demonstrate that the refractive index of the plasma may be lowered markedly by the injection of physiological saline solution without producing sig-

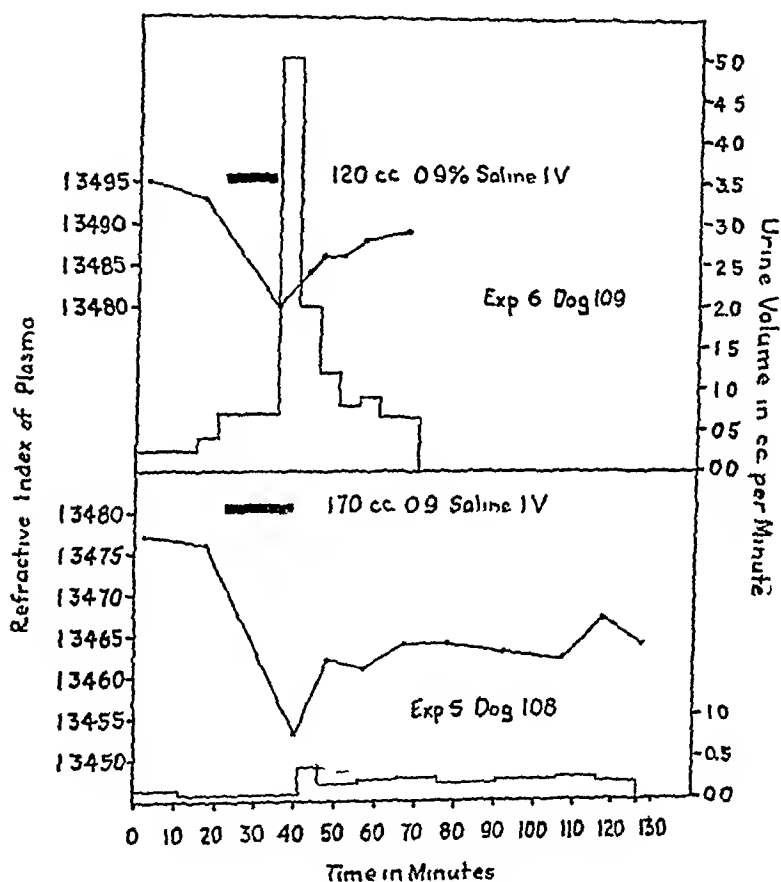


FIG 3B SALINE EXPERIMENTS 5 AND 6

nificant diuresis. This is additional evidence that the moderate decrease in the refractive index of the plasma observed in some of the salyrgan experiments played no significant rôle in the production of diuresis.

The refractive index of the plasma rises gradually during the early part of salyrgan diuresis. When the index has increased approximately 0.0010 no further concentration of the plasma occurs. Apparently sufficient fluid flows into the plasma from the tissue spaces to balance the loss by way of the kidneys.

The amount of water lost from the plasma may be calculated roughly from the change in the refractive index. Salyrgan Experiment 3 in which a comparatively moderate diuresis occurred may be used as an example. Dog 110 weighed 14 kilograms. If we estimate the plasma volume as 600 cc and assume a protein concentration of 6 grams per 100 cc, then an increase of 0.5 gram per cent in the protein concentration (a rise of 0.0010 in the refractive index) would mean a loss of approximately 50 cc of water from the plasma. The total amount of urine passed from the onset of diuresis to the end of the experiment was 105 cc. In Experiment 8 the urine exceeded the calculated loss of water from the plasma by approximately 150 cc.

The excess of urine volume over the calculated loss of fluid from the plasma points to an inflow of tissue fluids into the blood. The absence of hydremia preceding the onset of diuresis, the gradual rise of the refractive index of the plasma during the early part of diuresis and the maintenance of a level after the index has risen approximately 0.0010 indicate that this inflow is secondary to the loss of fluid from the blood by way of the kidneys. This secondary withdrawal of fluid from the tissue spaces is probably the mechanism by which the mercurial diuretics remove accumulations of fluid in the subcutaneous tissues and in the peritoneal cavity.

SUMMARY

The effect of salyrgan upon the water content of the plasma has been studied by means of the refractometer.

There is no evidence in these experiments that salyrgan diuresis is preceded by a mobilization of fluid from the tissue spaces.

The results point strongly to a primary direct action of salyrgan on the kidney with a secondary inflow of fluid from the tissue spaces to prevent excessive dehydration of the plasma.

BIBLIOGRAPHY

1. Saxl, P., and Heilig, R. *Ztschr f d ges exper Med* 1923 **xxviii**, 94. Über die Novasuroidiurese.
2. Bohn, Hans. *Ztschr f d ges exper Med*, 1923 **xxx**, 303. Experimentelle Studien über die diuretische Wirkung des Novasurols.
3. Bohn, Hans. *Deutsches Arch f klin Med* 1923 **cxi**, 225. Fortgesetzte Studien über Novasurol, seine Wirkung bei verschiedenen Lebensaltern und bei Diabetikern, sowie sein etwaiger Einfluss auf Ionenverschiebungen im Organismus.
4. Crawford, J. H., and McIntosh, J. F. *J Clin Invest* 1925 **i**, 333. Observations on the Use of Novasurol in Edema due to Heart Failure.
5. Mühling, A. *München med Wchnschr*, 1921, **lxviii**, 1447. Studie über die diuretische Wirkungsweise von Quecksilber. Ausgeführt mit dem organischen Quecksilberpräparat Novasurol.
6. Nonnenbruch, W., *München med Wchnschr*, 1921 **lxviii**, 1282. Ueber die Wirkung des Novasurols auf Blut und Diurese.

- 7 Bleyer, L, Klin Wchnschr, 1922, 1, 1940 Erfahrungen uber die Novasuroldiurese
- 8 Schmitz, H L, J Clin Invest, 1932, 11, 1075 Studies on the Action of Diuretics I The Effect of Euphyllin and Salyrgan upon Glomerular Filtration and Tubular Reabsorption
- 9 Robertson, T B, J Biol Chem, 1912, 11, 179 On the Refractive Indices of Solutions of Certain Proteins VI The Proteins of Ox-Serum, a New Optical Method of Determining the Concentrations of the Various Proteins Contained in Blood-Sera

STUDIES IN CONGESTIVE HEART FAILURE XXIII A
CRITICAL STUDY OF METHODS FOR DETERMINING
THE CARDIAC OUTPUT IN PATIENTS WITH
CARDIAC DISEASE

BY ARTHUR GROLLMAN BEN FRIEDMAN GURNEY CLARK AND
T R HARRISON

(From the Department of Medicine of Vanderbilt University Nashville)

(Received for publication April 26 1933)

The cardiac output of normal persons may be determined by methods the accuracy of which has been adequately demonstrated (4) In patients with congestive heart failure on the other hand so many factors intervene that the validity of a method applicable to normal individuals may no longer be assumed Hitherto comparatively little work has been done in testing the applicability of various cardiac output methods to patients with heart disease although data obtained by such methods may be subject to large errors

In the present investigation we have examined the accuracy of three methods of measuring the cardiac output and ascertained the extent of their applicability to subjects with congestive heart failure The Burwell and Robinson procedure (3), the venous plateau method (7), and the acetylene procedure (4) seemed to us to offer the greatest probability of proving applicable in heart disease The method of Burwell and Robinson depends upon the equilibration of the patient's blood with a gas mixture obtained by repeated rebreathings From a comparison of the oxygen content of this blood with that of a sample of arterial blood the arteriovenous difference is derived This method assumes that the same physicochemical equilibrium occurs between the blood and the gas obtained by the rebreathing procedure when shaken in a tonometer as occurs across the pulmonary epithelium during rebreathing Obviously this physiological equilibration occurs only slowly and may therefore lead to illusory constancy in any case in which pulmonary conditions inhibit (without totally preventing) normal oxygenation of the blood in the lungs Since such conditions are probably of frequent occurrence in cardiac disease, Burwell and Robinson, realizing these possible sources of error, limited their observations to individuals with normal lungs

In order to avoid the source of error in the Burwell Robinson procedure Harrison, Friedman and Clark (7) modified it Their venous plateau method depends upon the use of the lungs for the final equilibration

instead of a glass tonometer as in the Burwell and Robinson procedure. The patient rebreathes the final gas mixture, and during the rebreathing, samples of "mixed venous" blood are drawn from the femoral artery.

The acetylene procedure (4) is founded upon a principle entirely different from that of these two methods. It depends essentially upon measuring the rate of absorption of a foreign gas across the pulmonary epithelium.

Experiments demonstrating the sources of error occurring in these methods will be presented and the criteria which must be applied for demonstrating their validity in a given patient will be defined. When these criteria are satisfied accurate results may be obtained.

The attainment of homogeneous mixture in a lung-bag system in patients with congestive heart failure

The measurement of the cardiac output in patients with congestive heart failure is rendered difficult in all methods based upon equilibration of gas mixtures across the lungs by essentially two factors. The first of these is the change in the mechanical conditions in the lungs which makes it difficult to obtain samples of the gas mixtures which are in true equilibrium with the blood. A second factor is the involvement of the alveoli in a pathological process which prevents the establishment of true equilibrium (in a physicochemical sense) between the alveolar gases and the blood.

A prominent feature in patients with heart disease is the marked pulmonary alteration which mechanically interferes with any rebreathing procedure. The exact nature of the pulmonary lesions need not be described here, suffice it to say that loss of elasticity of the alveolar walls, congestion of the lungs, swelling of the pulmonary epithelium, the presence of edema in the alveoli and bronchioles, and, in some cases, emphysema, combine to increase the residual air and render difficult the attainment of a homogeneous mixture when a patient rebreathes a given gas mixture from a bag. If homogeneity of the contents of the lungs and of the bag is not attained it is obviously impossible to obtain a sample of gas from the bag which is representative of that in equilibrium with the blood.

The Burwell-Robinson, venous plateau and acetylene procedures are all dependent upon attaining complete mixture in a lung-bag system. It was, therefore, necessary to determine the length of time required for obtaining a homogeneous mixture in this system. For this purpose the method of Lundsgaard and Schierbeck (9), used previously by Grollman and Marshall (5), was utilized. The subject rebreathes a mixture of hydrogen in air under the same conditions as in the desired experimental procedure. By analyzing samples of the gas withdrawn simultaneously from the bag and from the mouth one can obtain an indication of the degree of homogeneity in the lung-bag system. The time required is

about fifteen seconds in normal individuals but is longer in subjects with abnormal lungs

There exists no definite and universally accepted nomenclature for describing succinctly the clinical condition of a given patient. The term "decompensation," for example may have a different denotation for different observers. We have included therefore in Table 1 data bearing on the clinical condition of the patients whom we have studied. Comparison of a result in the experimental tables with the clinical description of Table 1 will allow the reader to judge roughly concerning the conclusions to be drawn from the present study.

In Table 2 are given the results obtained in a series of patients showing various degrees of congestive heart failure as defined by the clinical data of Table 1. The patients rebreathed a mixture of hydrogen in air as is customary in measuring the cardiac output by the acetylene method. The volume of gas in the bag (Table 2, column 2) was that which could be inspired without difficulty by the patient (4). The number of respirations and the time after beginning rebreathing when collection of the samples was made are given in columns 3 and 4.

Since the respiratory quotient is less than unity during rebreathing (due to accumulation of carbon dioxide in the bag which prevents the usual loss of carbon dioxide from the mixed venous blood in the lungs) the hydrogen content of the sample obtained from the mouth is greater than that expired into the bag when homogeneous mixture has been attained in the lung bag system. A positive difference in the fifth column of Table 2 indicates therefore that mixture has occurred under the conditions of the rebreathing.

The data in Table 2 demonstrate the greater length of time necessary in patients with heart failure than in normal individuals for attaining homogeneous mixture between the air in the lungs and a gaseous mixture contained in a bag. Whereas normal subjects seldom require more than fifteen seconds, the subjects in Table 2 required eighteen seconds in two cases, twenty seconds in three cases, and twenty-five seconds or more in the remaining three. The application of these results to the problem of measuring the cardiac output in such cases will be given later.

Equilibration of gases across the pulmonary epithelium

The second possible source of error, which must be considered before applying to persons with congestive heart failure a method applicable to normal subjects, is the possible alteration of diffusion through the pulmonary epithelium due to pathological changes. If a change occurs in the lung which diminishes the rate at which a gas can pass into the blood from the alveolar spaces, physicochemical equilibrium may not occur and the method based upon the assumption that it does becomes inapplicable. Although much has been said concerning this possibility in studying cases

TABLE 1
Clinical data on subjects with organic cardiac disease studied in this investigation

Subject	Age	Sex	Race	Height	Edema free weight	Surface area	Type of cardiac disease	Degree of cardiac failure					Rales in lungs	Therapy being administered
								Degree of dyspnea		Vital capac- ity	Degree of edema	Degree of cardiac enlarge- ment		
								On exertion	At rest					
	years			inches	pounds	square meters				liters				
P N	59	M	Negro	67 5	158	1 82	Arteriosclerosis, hyper- tension	+	0	3 4	0	++	0	None
I M	28	F	White	66 5	121	1 62	Mitral stenosis and insuffi- ciency, auricular fibrillation	++	+	2 1	+	++	+	Digitalis Diuretics
E G	44	M	Negro	66 5	127	1 62	Syphilitic aortic insufficiency	++	±	2 7	0	++	±	Digitalis
F B	43	M	Negro	67 2	135	1 62	Syphilitic aortic insufficiency	++	++	2 5	++	++	++	Digitalis Diuretics
U R	37	M	Negro	68 2	132	1 70	Mitral stenosis, auricular fibrillation	+	0	2 9	0	+	0	Digitalis
P F	41	M	Negro	66 7	132	1 66	Syphilitic aortic insufficiency	++	±	2 8	±	++	±	Digitalis Diuretics
E B	54	M	Negro	67 2	135	1 69	Syphilitic aortic insufficiency	+	0	3 2	±	++	0	Digitalis
W C	69	M	White	68 5	132	1 70	Hypertension, arteriosclero- sis, emphysema	++	+	2 4	+	++	++	Digitalis Diuretics
M Y	74	M	White	66 0	105	1 51	Arteriosclerosis, emphysema	++	±	2 6	+	++	+	Digitalis
M S	39	F	White	66 0	126 5	1 62	Syphilitic aortic insufficiency	++	0	3 1	0	++	++	Digitalis

TABLE 2

The time necessary for attaining homogeneous mixture in a lung bag system

Subject	Volume in bag	Number of respirations	Duration of rebreathing	Difference between hydrogen concen- tration in alveolar sample and in bag	Time necessary for attaining homogeneous mixture
P N	liters 1.8	7 10 11 13	seconds 18 20 21 23	per cent of hydrogen 0.00 +0.05 +0.03 +0.03	seconds 20
I M	1.2	7 6 8 8	17 18 22 25	-0.03 +0.02 +0.08 +0.01	18
E G	1.5	7 7 7 9	16 18 21 25	-0.15 -0.00 +0.01 +0.06	20
F B	1.5	9 10 12 13	18 20 23 26	-0.09 -0.02 -0.05 +0.05	25
U R	1.5	8 9 10	17 18 20	-0.06 +0.00 +0.01	18
P F	1.2	9 8 8 9	19 23 26 28	-0.10 -0.12 0.00 0.00	26
E B	1.3	7 8 10 10	16 19 21 23	-0.04 0.00 +0.01 +0.01	20
W C	1.2	15	50	-0.10	Incomplete mixture

of heart failure, it is ordinarily relatively less important than the factor of inadequate mixture, which has just been discussed.

The influence of a pathological condition on diffusion through the lung can be obtained from an analysis of the degree of oxygen saturation of the arterial blood. The fact that significant anoxemia occurs only in severe cases of congestive heart failure is an indication that this is a result only of extreme modifications from normal.

It is necessary to consider the possibility of failure to obtain a true equilibrium in cases in which oxygen deficiency of the arterial blood exists.

In the "oxygen Fick" methods in which it is necessary to secure equilibrium between the gas in the blood and that in a bag, the difference in tension between the two may not be great. Nevertheless any impediment to the passage of oxygen across the pulmonary epithelium may lead to erroneous results. It was this possibility which led Burwell and Robinson to apply their method to normal individuals only.

One of the fundamental assumptions of the acetylene method is the occurrence of true physicochemical equilibrium between this gas in the alveoli and in the blood. That equilibrium occurs in persons with normal circulatory systems and even in some cases of decompensated heart disease was demonstrated by Baumann and Grollman (2) and by Grollman, Proger, and Dennig (6). In one case (senile emphysema with chronic congestive failure) the former authors failed to observe its occurrence. This failure was attributed to an inability to obtain mixture in the lung-bag system and was therefore apparent and not real. Unless one obtains an actual alveolar sample, one cannot judge whether equilibrium has occurred in the lungs by comparison of the gas obtained with a sample of arterial blood. So far as measurement of the cardiac output is concerned, the question is purely academic whether equilibrium has occurred or whether failure to attain it is due to failure to obtain a true alveolar sample. Regardless of the cause, the method is inapplicable in such a case.

In Figures 1, 2, 3, and 4 are reproduced typical experiments demonstrating the relation of the concentrations of acetylene in the blood and in the gas mixtures rebreathed. Two patients (Figs 1 and 2) rebreathed an acetylene-air mixture from a rubber bag as is usual in measuring the cardiac output. At certain intervals samples of arterial blood and a gas sample from the bag were collected and their acetylene contents determined as described by Grollman, Proger, and Dennig (6). It will be noted in

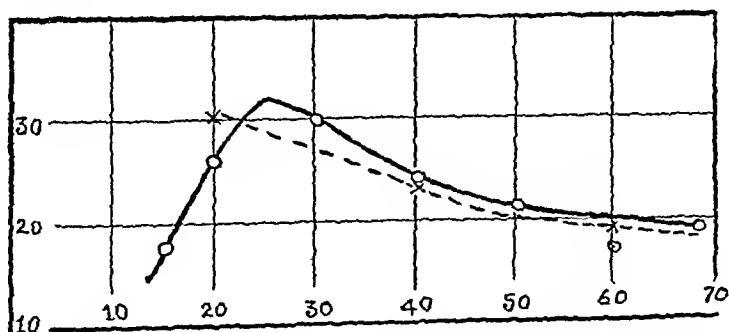


FIG 1 A COMPARISON OF THE ACETYLENE CONTENT OF THE ALVEOLAR AIR AND THE ARTERIAL BLOOD WHILE REBREATHING FROM A RUBBER BAG. SUBJECT E G (SEE TABLE 1)

Ordinates represent the acetylene tension in millimeters of mercury, the abscissae represent the time in seconds after beginning the rebreathing

○ Blood samples

× Gas samples

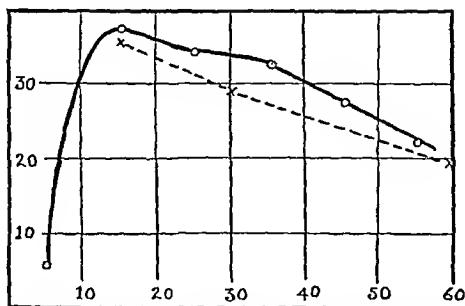


FIG 2 A COMPARISON OF THE ACETYLENE CONTENT OF THE ALVEOLAR AIR AND THE ARTERIAL BLOOD WHILE REBREATHING FROM A RUBBER BAG SUBJECT J M (SEE TABLE 1)

Ordinates represent the acetylene tension in millimeters of mercury, abscissae the time in seconds after beginning the rebreathing

○ Blood samples

× Gas samples

Figure 1 that there is complete concordance between the amounts of acetylene in the blood and in the bag at twenty seconds after beginning the rebreathing and thereafter. Since this subject could not mix in a period of time less than twenty seconds as seen in Table 2, the low content of acetylene in the samples of blood collected at fifteen and twenty seconds is to be attributed not to failure to attain equilibrium between the acetylene of the alveoli and the blood, but to the fact that it required twenty seconds to establish a uniform concentration of gas in the lung bag system. The remainder of the curve is evidence against the existence in this patient of

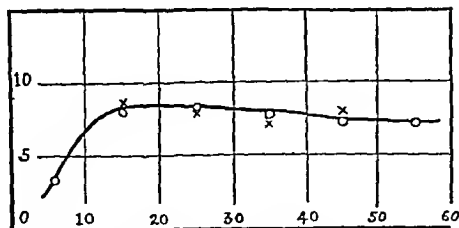


FIG 3 A COMPARISON OF THE ACETYLENE CONTENT OF THE ALVEOLAR AIR AND THE ARTERIAL BLOOD WHILE REBREATHING FROM A SPIROMETER SUBJECT I M (SEE TABLE 1)

Ordinates represent the acetylene tension in millimeters of mercury abscissae the time in seconds after beginning the breathing

○ Blood samples

× Gas samples

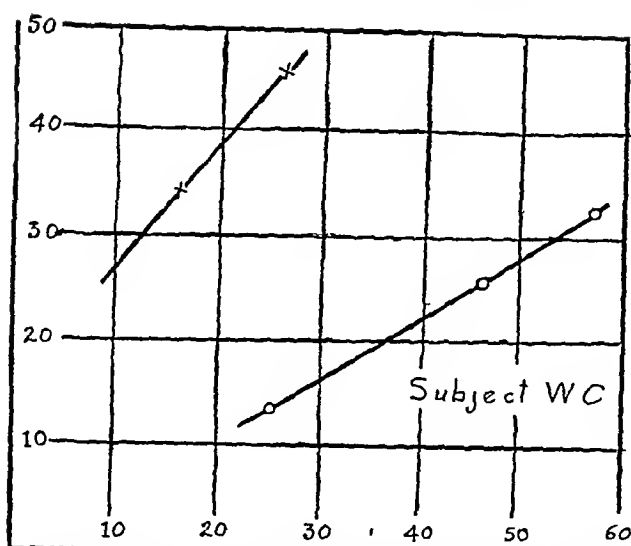


FIG 4 A COMPARISON OF THE ACETYLENE CONTENT OF THE "ALVEOLAR" AIR AND THE ARTERIAL BLOOD WHILE BREATHING FROM A SPIROMETER SUBJECT W C (SEE TABLE 1)

Ordinates represent the acetylene tension in millimeters of mercury, abscissae, the time in seconds after beginning the breathing

○ Blood samples

× Gas samples

an impediment to the passage of acetylene across the pulmonary epithelium which might interfere with the attainment of equilibrium between the gas in the lungs and in the blood

The curves of Figure 2 were obtained similarly to those of Figure 1. At five seconds, the acetylene concentration in the blood was very low, for insufficient time had elapsed since beginning of rebreathing to allow entrance into the alveoli of sufficient amounts of acetylene. After fifteen seconds mixture in the lung-bag system occurred so that the acetylene content of the blood and of the alveoli was parallel. The concentrations in the blood were higher, however, due to the fact that it requires five to ten seconds for the blood to travel from the lungs to the peripheral artery from which it is drawn. If one allows for this (2, 6), there results an identity of the curves of the concentrations in the alveoli and in the blood which demonstrates the existence of true physicochemical equilibrium. In Figure 1, there is also a tendency for the acetylene concentrations to be higher in the blood than in the alveoli but in this case the more gradual slope of the curve prevents this from manifesting itself prominently in the samples collected after forty seconds.

The studies of Figures 1 and 2 as well as previous ones of Grollman and his coworkers require the time necessary for the passage of the blood from the lungs to the peripheral artery to be taken into consideration. As is evident from the curves of Figures 1 and 2 and from the results of Baumann and Grollman (2), this possible source of error is negligible.

being only one or two per cent. This smallness results from the relatively small drop in acetylene concentration which is taking place in the bag (Figs 1 and 2). The assumption involved need not be made, however, if a constant alveolar concentration is maintained. This is accomplished by having the subject inspire at a constant rate from a spirometer containing an acetylene air mixture. At given intervals forced expirations are made of which alveolar samples are collected. The results of such an experiment on I M are given in Figure 3. The concentrations of acetylene in the alveoli and in the blood lie on a uniform curve after fifteen seconds the time necessary for substituting the gas mixture for the air in the lungs.

Patients I M and E G were both cases of congestive heart failure (Table 1) and in them apparently the transport of acetylene across the lungs was sufficiently rapid to ensure equilibrium, despite the fact that there was a slight degree of arterial anoxemia in both subjects. The greater ease of attaining equilibrium between the blood and alveoli in the case of acetylene in comparison with oxygen, is what one might predict on strictly physical grounds. Indeed, it may be said (in view of the experiments just cited and those previously reported) that it is improbable that interference with diffusion in the lung is ever the limiting factor of the acetylene method. Because of its high solubility and its small molecular weight the tension of acetylene in the blood is the same as that in the alveoli to which this blood is exposed. But if conditions exist in which part of the lungs are fibrosed, consolidated collapsed or so markedly edematous as to prevent its entrance and, therefore, the passage of acetylene into the blood, the acetylene method cannot measure the blood flowing through these portions of lungs.

One encounters subjects in whom analysis of the acetylene content of the blood shows a smaller concentration than is present in the supposed "alveolar" air. An example is shown in Figure 4 which illustrates the lack of relation between the acetylene contents of the blood and of the "alveolar" samples. In another similar experiment the same patient inspired a mixture of gas, the acetylene tension of which was 54.4 mm, but whose arterial blood after breathing 55 seconds contained only 29.4 mm. Mixing as tested on this patient (W C Table 2) was incomplete even after 50 seconds rebreathing. The discordance in the acetylene content of "lung air" and blood as observed in this patient cannot be attributed therefore to any impermeability of the pulmonary epithelium but is due to an inability to obtain a true alveolar sample.

The Burwell Robinson procedure

On the basis of the experiments already cited we can define the conditions under which a given cardiac output method based on respiratory maneuvers is applicable and the methods whereby its applicability can be tested.

The Burwell-Robinson procedure consists essentially in rebreathing repeatedly from a bag in order to obtain a gas in equilibrium with the mixed venous blood. Not only must mixture in the lung-bag system be ensured but sufficient time must be allowed thereafter for the blood to alter the composition of the gas in the bag. The circulation time cannot be exceeded and hence one is limited to a period of time about thirty seconds (in cases of congestive heart disease) in which to carry out the rebreathing. The process can be carried out repeatedly, however, and in this way one may in normal individuals finally obtain a mixture in true equilibrium with the mixed venous blood. Unfortunately, as has previously been indicated (4), constancy of the final mixture cannot (as has often been done in the past) be taken as a proof of equilibration. A patient with a large volume of residual air can replace the air in his lungs partially during the preliminary rebreathing and dilute the gas in the bag to a certain extent only. This was the source of error in a number of earlier methods.

TABLE 3
The oxygen content of two bags during alternate rebreathings
Subject P F (Table 1)

Number of rebreathings	Oxygen content	
	Bag 1	Bag 2
	<i>per cent</i>	<i>per cent</i>
4		3.76
5	4.95	
6	4.87	
8	4.40	
9	4.17	
11	4.1	3.85
12	4.15	

The experiment cited in Table 3 demonstrates how delusive constancy of the composition of the bag mixture may be. The patient in this experiment (P F, Table 1) rebreathed alternatively from bags 1 and 2 as in the Burwell-Robinson method, the bags initially having contained mixtures of different oxygen contents. After nine rebreathings the oxygen content of bag 1 was constant and equal to 4.16 per cent. Bag 2, on the other hand, was little affected after the fourth rebreathing and had an oxygen content even after the eleventh rebreathing of only 3.85 per cent. Both of these bags could obviously not be in equilibrium with the mixed venous blood and hence the supposition of their equilibration with the patient's blood might lead to an erroneous result. It must be remembered also that due to the steepness of the oxygen dissociation curve of blood at the degree of oxygen saturation of the mixed venous blood even slight differences in oxygen tension cause a considerable difference in the oxygen content from which, in turn, is calculated the cardiac output.

The extent to which the source of error just described may invalidate attempts to obtain a gas mixture in equilibrium with the mixed venous blood is further demonstrated in Table 4. In this experiment the patient,

TABLE 4

Changes in the oxygen content of the bag during rebreathings in a patient with congestive heart failure (E G Table 1)

Experi- ment number	Number of wash breaths	Time for washing	Sample 1		Sample 2		Sample 3	
			Time of collection	Oxygen content	Time of collection	Oxygen content	Time of collection	Oxygen content
		<i>seconds</i>	<i>seconds</i>	<i>per cent</i>	<i>seconds</i>	<i>per cent</i>	<i>seconds</i>	<i>per cent</i>
1	1	5	20	4.27	24	4.27	28	4.19
2	2	5	20	3.99	25	3.99	30	3.91
3	3	7	27	3.23	30	3.30	34	3.21
4	4	7	23	1.41			28	3.56
5	4	9	29	3.38	34	3.28	38	3.34

E G, rebreathed from a bag containing 12 liter of nitrogen. Samples were taken at various times during rebreathing. The number of the preliminary "wash" breaths were varied (column 1) in order to vary the oxygen contents of the lung bag system in the different experiments. The results demonstrate the marked effect which preliminary washings have on the final composition of the gas in the bag. This was observed also by Burwell and Robinson (3). The results also demonstrate the extremely small changes in oxygen content of the bag which can occur during the breathing of mixtures varying as much as one per cent in composition. The rise in oxygen content (in the 3d experiment) and the absence of a decrease (in the first and second experiments) of the oxygen content between the collections of the first and second samples are due to inadequate mixture in the lung-bag system. The times in the 5th, 7th and 9th columns refer to that which elapsed after beginning the washing. Actual mixture occurs only after the beginning of rebreathing and hence fifteen seconds (in the first and second experiment), or sixteen seconds (in the 4th experiment), do not suffice for attaining homogeneous mixture in the lung bag system at the time of collection of the first sample from the bag.

In order to be certain that the value of a given experiment is not destroyed by the illusive constancy described, it is necessary to approach the final equilibrium with various oxygen contents in the bag. After preliminary rebreathing the patient should rebreathe a given mixture and two samples should be taken after adequate mixture has been attained and before recirculation has begun. It may be advisable to measure the oxygen content with an accuracy greater than ± 0.01 per cent in order to detect small changes such as are masked in Table 4 in which the results are accurate only to about ± 0.05 per cent.

Another procedure which may possibly avoid the source of error under discussion is one which was employed by Burwell and Robinson. Their subjects rebreathed alternately into two bags, the one having an oxygen tension below and the other an oxygen tension above that of the mixed venous blood. In their normal subjects the oxygen tension of the two bags became identical. We have verified their observations in several patients with cardiac disease, but have found others, especially cases of aortic insufficiency, in whom the final values differed markedly as in Table 3. Obviously the results obtained by this method, in patients with heart failure, are open to grave errors unless one ensures the absence of all the vitiating factors just described. Other so-called "Fick" oxygen or carbon dioxide methods are even more liable to error in such patients.

The venous plateau method

In applying this method one must consider the possible sources of error outlined in connection with the Burwell-Robinson method in obtaining the gas mixture used in the final rebreathing.

In drawing blood from the femoral artery certain precautions must, however, also be observed. In the first place ample time must elapse to allow mixture between the gas in the bag and in the lungs to take place during the final rebreathing. Samples of blood must be drawn either before or within ten seconds after the patient stops rebreathing, otherwise mixture of partially aerated blood with mixed venous blood may give a plateau which is not representative of the true mixed venous blood. Nor can the patient continue to rebreathe after the completion of a circulation time unless the composition of the rebreathed gas be maintained constant, for example, by allowing the patient to breathe from a spirometer containing gas identical in composition with that in the bag after the final rebreathing.

Despite the theoretical applicability of this method for permitting the prolongation of the rebreathing time, it is impracticable because anoxemia (particularly in patients with congestive heart disease) causes extreme discomfort and may initiate an attack of cardiac pain in persons with coronary disease or aortic insufficiency. Anoxemia may in fact cause marked reduction in cardiac output with consequent prolongation of the circulation time in diseased hearts. Blood samples taken more than five to ten seconds after cessation of the rebreathing may therefore give a plateau which in no way represents that of the mixed venous blood. The plateau corresponding to the mixed venous blood, to be reliable, must occur in the blood of the femoral artery about ten seconds after adequate mixture in the lung-bag system has occurred. Before this time the mixed venous blood will lose oxygen if the patient has been "overwashed" in the preliminary "wash breaths" or will take it up if this washing has been insufficient. Only after homogeneous mixture has occurred does the blood reflect the

composition of the gas mixture in the bag. Correlation of the time at which an oxygen plateau in the blood occurs with that of the stage of re-breathing is, therefore, of great importance.

A more detailed discussion of the possible sources of error in the Burwell-Robinson and venous plateau methods will be given in a future publication (7).

The acetylene method

The validity of the underlying assumptions of the acetylene method has been described in detail elsewhere (4) and need not be repeated. In applying the method to cases with congestive heart failure one must consider the three fundamental requirements of the method: (1) Do the samples taken for analysis represent the gas which is in equilibrium with the blood traversing the lungs? (2) Does the gas in the alveolar air enter into equilibrium with the blood? (3) Is the re-breathing procedure completed before the time of a complete circulation of the blood?

As shown in Table 2 it is necessary to prolong the preliminary re-breathing of the acetylene mixture longer in cases of congestive heart failure than in normal individuals before taking the first sample. If the sample is taken before mixture occurs the resultant cardiac output will be false and may be as much as fifty per cent greater or less than the true value. The question of attaining equilibrium across the pulmonary epithelium as already stated seldom, if ever, is involved in applying the acetylene method. Care must be taken, however, not to continue re-breathing beyond a circulation time, otherwise the estimate of the cardiac output will be too low.

In applying the acetylene method in congestive heart failure how can its applicability be tested without undertaking the laborious work of determining the various factors just outlined? Taking three samples for analysis instead of two suffices for this purpose. The apparatus previously described (4) has been altered to provide another capillary tube which permits collection of the third sample. In cases of congestive heart disease the samples are taken preferably at 20, 25, and 30 seconds after beginning the re-breathing. The samples taken at 20 and 25 seconds are used to calculate one value and those taken at 25 and 30 are used for another. If these agree within the limits of the experimental technical errors one is assured that the assumptions underlying the applicability of the method are valid and that the result is accurate. In all measurements this procedure, involving as it does little extra work, is suggested even in cases in which the applicability of the method can be taken for granted.

In Figure 5 is reproduced an experiment illustrating these points. The patient (P N, cf Table 1) could bring mixture about, as shown in Table 2, in twenty seconds. Samples collected at 18 and 23 seconds gave an apparent cardiac output of 4.25 liters, a value too high because the first sample was taken at 18 seconds when mixture was not quite complete and,

had, therefore, a higher content of acetylene than was present in the alveoli and in equilibrium with the blood. When samples were taken at 20 and 25 seconds and at 25 and 30 seconds, values were obtained which agreed within the limits of the accuracy of the analytical methods employed. The average of these values (3.8 liters) was the correct cardiac output of this patient under the condition of the experiment. When the samples were taken at 30 and 35 seconds, the estimation was too low (3.1 liters), because blood containing acetylene had returned to the lungs. In the cases of congestive heart failure which we have studied, recirculation was evident after 30 seconds. This circulation time as observed in these pathological cases is longer than that observed (4) in normal persons due to the greater velocity of the normal blood flow.¹

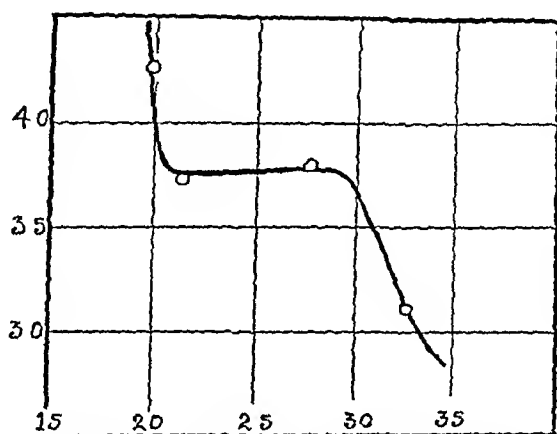


FIG 5 THE EFFECT OF VARYING THE TIME OF SAMPLING ON THE APPARENT CARDIAC OUTPUT AS OBTAINED BY THE ACETYLENE METHOD. SUBJECT P N (NOT BASAL)

The ordinates represent the apparent cardiac output in liters per minute, the abscissae the average time of collection of the two samples used in determining the cardiac output. The plateau represents the true cardiac output.

Comparison of results by the different methods

The diversity in principles upon which the acetylene method (foreign gas principle) and the Burwell-Robinson and venous plateau methods (oxygen Fick principle) are based gives importance to a comparison of results obtained by these methods when applied to the same patient. In a previous paper, Harrison, Friedman and Clark (7) have shown the excellent

¹ All discussions of the circulation time in the present paper refer obviously only to man. It is well known (4) that the relative circulatory rates in the smaller mammals is greater than it is in man. Hence it is not surprising that Starr and Collins (*Am J Physiol*, 1933, civ, 650) should have found an appreciable return of blood to the heart in 15 seconds in dogs. Their conclusions are obviously inapplicable to man.

agreement obtained by the various methods in normal subjects. This may be taken as added evidence for the accuracy with which the cardiac output in normal subjects may be measured by these methods. In Table 5 are

TABLE 5

A comparison of results obtained by the Burwell Robinson venous plateau and acetylene methods as applied to patients with congestive heart failure

Subject	Oxygen consumption	Acetylene method		Burwell Robinson method		Venous plateau method	
		Arterio-venous oxygen difference	Cardiac output	Arterio-venous oxygen difference	Cardiac output	Arterio-venous oxygen difference	Cardiac output
	cc per minute	cc per liter	liters per minute	cc per liter	liters per minute	cc per liter	liters per minute
U R	198	90	2.2	94	2.1	90	2.2
M Y	170	50	3.4	50	3.4	50	3.4
E B	233	71	3.3	61	3.8	68	3.4
M S	215	65	3.3	60	3.5	67	3.2
P F	212	60	3.6	57	3.7	62	3.4
E G	263	81	3.2				
P N	258	69	3.7				
I M	180	84	2.1				

given a series of results obtained in patients with congestive heart disease. The procedures were performed at one sitting. The results agree within the limits of the experimental errors involved and add further evidence of the validity of the theoretical considerations now discussed and the accuracy of the results obtained.

Relatively few values of the cardiac output in congestive heart failure are to be found in the literature in which the necessary precautions have been taken to demonstrate the applicability of the method to the patient under investigation. In view of the small number of cases which we have studied (Table 5) no final conclusion concerning the cardiac output in cardiac disease seems warranted. Of the eight patients cited in Table 5, the cardiac index (4) is reduced in five and within normal limits in the remainder. This does not necessarily imply, however, that the blood supply to the tissues in these cases is adequate, for under the disturbed conditions in the body, particularly in the presence of edema (8), the normal blood supply may still be inadequate for proper supply of the metabolic requirements of the tissues. During exertion, moreover the cardiac output in these patients may be markedly reduced from the normal (1). We have not yet found patients with congestive failure and abnormally great cardiac output.

Choice of methods

As to the relative merits of using one or another of the methods studied, the laboriousness of the Burwell-Robinson and venous plateau methods

both to the patient and to the investigator renders them inferior to the relatively simple acetylene procedure. In persons with normal arterial oxygen saturation the method of taking three samples, as advocated above, for the acetylene method should exclude the possibility of error. With care, moreover, the method will be found to be applicable to a large percentage of subjects with advanced cardiac disease and with mild congestive failure. In those subjects in whom the method is not applicable, the venous plateau method may be applied. Unfortunately, it is in these very cases that the pitfalls inherent in the latter method occur and great care and labor are required to avoid them. A further study of these cases is contemplated.

SUMMARY

A critical study has been made of the possible sources of error of the Burwell-Robinson, venous plateau and acetylene methods for measuring the cardiac output which may invalidate the results obtained when applying them to patients with congestive heart failure. The methods to be used for detecting and avoiding these errors are indicated. Results as obtained by these different methods are given.

It is a pleasure to express our appreciation to Dean W. S. Leathers for interest in the work and for a grant of money which made our collaboration possible. We are also indebted to Dr. C. S. Burwell for his helpful criticism and advice during the present study.

BIBLIOGRAPHY

- 1 Banst, H. W., and Groscurth, G., *Ztschr. f. Kreislaufforsch.*, 1930, **xii**, 657. Die Kreislauleistung wahrend und nach der Arbeit beim Gesunden und kranken Menschen.
- 2 Baumann, H., and Grollman, A., *Ztschr. f. klin. Med.*, 1930, **cxv**, 41. Über die theoretischen und praktischen Grundlagen und die klinische Zuverlässigkeit der Acetylenmethode zur Bestimmung des Minutenvolumens.
- 3 Burwell, C. S., and Robinson, G. C., *J. Clin. Invest.*, 1925, **i**, 47. A Method for the Determination of the Amount of Oxygen and Carbon Dioxide in the Mixed Venous Blood of Man.
- 4 Grollman, A., *The Cardiac Output of Man in Health and Disease*. Charles C. Thomas, Publisher, Springfield, Ill., 1932.
- 5 Grollman, A., and Marshall, E. K., Jr., *Am. J. Physiol.*, 1928, **lxxxvi**, 110. The Time Necessary for Rebreathing in a Lung-Bag System to Attain Homogeneous Mixture.
- 6 Grollman, A., Proger, S., and Dennig, H., *Arch. f. exper. Path. u. Pharmacol.*, 1931, **clxi**, 463. Zur Bestimmung des Minutenvolumens mit der Acetylenmethode bei Arbeit bei normalen und kranken Menschen.
- 7 Harrison, T. R., Friedman, B., and Clark, G. In press.
- 8 Harrison, T. R., and Pilcher, C., *J. Clin. Invest.*, 1930, **viii**, 259. Studies in Congestive Heart Failure. I. The Effect of Edema on Oxygen Utilization.
- 9 Lundsgaard, C., and Schierbeck, K., *Am. J. Physiol.*, 1923, **lxiv**, 210. Studies on the Mixture of Air in the Lungs with Various Gases. I.

PHYSIOLOGICAL DISTURBANCES DURING EXPERIMENTAL DIPHTHERITIC INTOXICATION I BLOOD SUGAR, LACTIC ACID AND NON-PROTEIN AND AMINO-ACID NITROGEN¹

By HERMAN YANNET AND DANIEL C DARROW

*(From the Department of Pediatrics Yale University School of Medicine
New Haven)*

(Received for publication May 20 1933)

The therapy of diphtheria and control of the disease is largely based on outstanding achievements in the field of immunology, starting with von Behring's discovery of antitoxin, going on to Schick's method of measuring susceptibility to toxin and culminating in the development of means of producing active immunity by injection of a toxin antitoxin mixture. Although the administration of antitoxin constitutes the chief mode of treating diphtheria, in a considerable number of severe cases this therapy alone is inadequate. It was believed, therefore, that additional study of the metabolic disturbances of diphtheritic intoxication was indicated and might supply data which would suggest additional lines of treatment. This and three subsequent papers (29, 30) will report studies in this field.

Disturbances in carbohydrate metabolism during experimental and clinical diphtheritic intoxication have been the subject of intensive investigation. In 1914, Rosenthal (1) demonstrated delayed sugar tolerance curves in rabbits following the injection of diphtheria toxin. This was confirmed by other investigators (2, 3, 4). With doses of toxin which lead to death in two to three days Rosenthal and others (4, 5, 6) frequently found markedly reduced fasting blood sugar values 12 to 24 hours before the death of the animals. Lawrence and Buckley (7), injecting smaller doses of diphtheria toxin (causing death in from 6 to 8 days), noted the occasional occurrence of a pre lethal rise in the blood sugar. Schwentker and Noel (6) demonstrated a similar tendency towards hyperglycemia in the less severely intoxicated animals. They found a fairly close direct relationship between the period of survival and the elevation of the blood sugar. Impairment of the normal blood sugar depressing action of insulin was demonstrated in rabbits poisoned with diphtheria toxin by Lawrence and Buckley (7) and by Netzeley (8). Sweeney (4), on the other hand, could find no diminution in the response to insulin, under similar conditions.

Investigations during the course of diphtheria in humans yielded results which are similar in many respects to those cited above. Hector (9), in 1926, found delayed sugar tolerance curves in 5 of 6 patients. These tests when repeated during convalescence were all normal. Similar findings were reported

¹ This work was aided by a grant from The Corn Products Refining Company

by Elkeles and Heimann (10), Prochajka (11), Schwentker and Noel (6), Benn, Hughes and Alstead (12) and Brems (13). In occasional patients the fasting blood sugar values were found moderately elevated (9, 10, 13), in one patient reported by Elkeles and Heimann, as high as 400 mgm per cent. The latter authors also reported extremely low blood sugar values in some of their patients. The injection of insulin prior to the administration of glucose for the sugar tolerance tests was found by Elkeles and Heimann to have no appreciable effect upon the resulting curve. Schwentker and Noel, on the other hand, found that the injection of insulin definitely altered the response in the direction of the normal sugar tolerance curve.

The occurrence of glycosuria during the clinical course of diphtheria has been noted. Hibbard and Morrissey (14) found reducing substances in the urine of 34 of 96 patients and in 8 of 9 fatal cases. The glycosuria in the non-fatal cases was transitory, disappearing during convalescence. Brems (13) also found spontaneous glycosuria in some of his patients. Elkeles and Heimann, on the other hand, failed to find urinary sugar in any of the cases, despite the presence of hyperglycemia.

The bearing of the above investigations upon the nature of the disturbance in carbohydrate metabolism during diphtheritic intoxication has been the subject of much speculation. The suggestions that have been offered fall principally into two groups, namely (1) a disturbance in the adrenals or related glands of internal secretion, (2) a depression or dysfunction of the insulin mechanism. Rosenthal (1), Mikami (5) and Elkeles and Heimann (10) favored the former concept of adrenal damage, although Rosenthal felt that liver injury played an important but secondary rôle. Lawrence and Buckley (7), on the basis of histological evidence of injury to the thyroid and adrenals, implicated the latter glands as the responsible factors. Hector (9) considered that the basis for the disturbance lay in a derangement of the entire endocrine apparatus as well as moderate liver injury. The insulin mechanism was believed primarily responsible for the disturbances of carbohydrate metabolism by Sweeney (4), Sweeney and Lackey (3), Schwentker and Noel (6) and Benn, Hughes and Alstead (12). The latter two groups of investigators utilized insulin and glucose as an adjunct to the antitoxin treatment of the disease with apparently good results.

The literature on the nonprotein nitrogen constituents of the blood is less voluminous. In 1914, Karsner and Denis (15) found, following the injection of diphtheria toxin into cats, a slight increase of the nonprotein nitrogen during the first 2 days and then a marked rise up to 200 mgm per cent 1 or 2 days before death (4 to 6 days). They attributed these results to the development of a nephritis, tubular in nature, during the early phase of the intoxication and vascular during the latter phases. Glesinger-Reischer and Glesinger (16) found the blood urea nitrogen greater than 30 mgm per cent in 16 of 21 patients with diphtheria and over 50 mgm per cent in seven. Following the administration of antitoxin, 20 to 50 per cent reductions in the urea values occurred in about half of the patients. Because of the essentially negative urinary findings they attributed the elevations in blood urea to the toxic destruction of body proteins. Prochajka (11) found that the nonprotein nitrogen of the blood was a most valuable prognostic sign in diphtheria patients. From a study of 86 cases he cited the following statistics: (1) when the nonprotein nitrogen was less than 40 mgm per cent, the mortality was 5 per cent, (2) when the nonprotein nitrogen was between 40 and 50 mgm per cent, the mortality was 32 per cent, (3) when the nonprotein nitrogen was over 80 mgm per cent, the mortality was 69 per cent.

In experimental animals and patients with diphtheria Lereboullet, Gournay and Donato (17) found elevated blood urea but no disturbance in elimination of phenolsulphonaphthalein nor marked histological changes in the kidneys. They emphasized the fact that the degree of azotemia was directly proportional to the severity of the diphtheritic intoxication and believed that the elevation in blood urea was brought about by a suprarenal disturbance. It is important to point out here that the phenolsulphonaphthalein excretion test becomes difficult of interpretation in the presence of the severe liver damage that is usually found in diphtheritic intoxication. Hanner and Whipple (18) showed that liver injury produced by chloroform and phosphorus poisoning was accompanied by a definite increase in the renal excretion of the dye. A normal excretion during diphtheria therefore cannot be used as an indication of normal renal functions. Derot (19) demonstrated, in rabbits injected with diphtheria toxin that the rise in blood nonprotein nitrogen was accompanied by a roughly parallel rise in blood creatinine.

No reports of amino acid determinations in experimental diphtheritic poisoning or clinical diphtheria have been found in the literature.

PROCEDURE AND METHODS

Rabbits weighing from 1400 to 2000 grams were employed in this investigation as the experimental animals. The diet for approximately one week prior to the experimental period consisted of a liberal supply of greens and oats. All food was removed from the cages 24 hours before the injection of the diphtheria toxin, and none was given during the balance of the experimental period. Water was supplied in liberal quantity. The diphtheria toxin used was found to contain 62 minimal lethal doses per cubic centimeter. The toxin was diluted with sterile saline so that the calculated dose was contained in about 0.3 cc of solution. This was injected intravenously.

One series of animals received approximately three quarters, and another series two to three minimal lethal doses. The smaller dose caused the death of the animals in from 4 to 7 days, the larger dose, in from 1 to 3 days. The animals were bled by cardiac puncture every day or every other day, depending on the severity of the intoxication. About 5 cubic centimeters of blood were drawn off and transferred to a bottle containing sufficient potassium oxalate to prevent clotting. Each bottle also contained a small quantity of powdered sodium fluoride to inhibit glycolysis. The Folin Wu method of precipitation was used to obtain a protein free filtrate.

For the blood sugar determinations the Shaffer, Hartmann and Somogyi (20) method was employed. Nonprotein nitrogen was determined colorimetrically by direct Nesslerization following digestion of the protein free filtrate with sulphuric acid and 30 per cent hydrogen peroxide. Lactate was determined by the method of Avery and Hastings (21).

Amino acid nitrogen was determined by Folin's colorimetric method (22). It was found using the quantities prescribed by Folin, namely, 5 cc of the 1:10 filtrate and 1 cc of the 0.5 per cent solution of sodium β naphthoquinonesulfonate, that the largest concentrations of amino-acid nitrogen which could be determined with any degree of accuracy approximated from 6 to 10 mgm per 100 cc of blood. Schmidt (23) made this same observation in a paper published in 1929. It was found most practicable in cases where there was doubt as to the approximate concentration of the amino acid nitrogen to set up a series of 3 tubes for each specimen of blood containing 1, 3 and 5 cc respectively of the filtrate and to add 2 cc of the reagent to each. The tube in which the color

that developed was most closely matched by the standard, was used for the colorimetric comparison. The error in the method is approximately 5 per cent in the lower values and 10 per cent when dealing with values in the vicinity of 40 to 60 mgm per cent.

RESULTS

The results are summarized in Tables I and II, and representative experiments are graphically presented in Charts 1 and 2. It is evident that the data confirm the findings of previous investigators as to the changes that occur in the blood nonprotein nitrogen and sugar during the experimental intoxications. Briefly, the results can be described as follows:

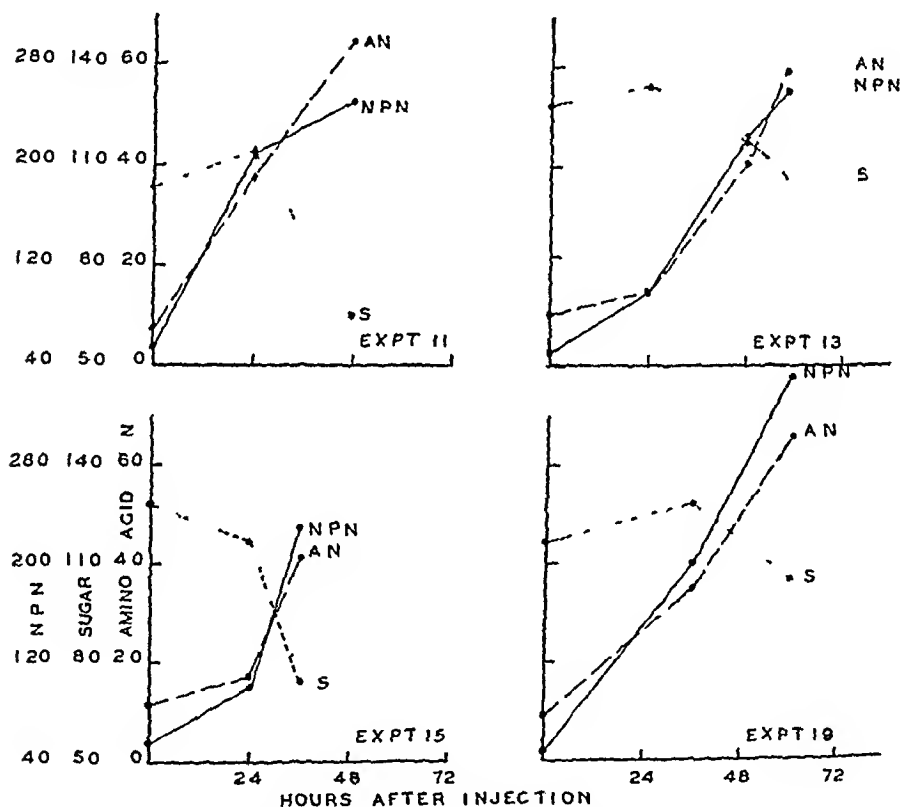


CHART 1 CHANGES OF BLOOD SUGAR, NONPROTEIN NITROGEN AND AMINO-ACID NITROGEN FOLLOWING THE INJECTION OF 3 MINIMAL LETHAL DOSES OF DIPHTHERIA TOXIN

After a relatively large dose of toxin, i.e., one causing death in less than 3 days, there is a progressive increase in the nonprotein nitrogen which can be demonstrated as early as 24 hours after the injection, a definite tendency for the blood sugar to fall, in some cases, to relatively low levels, a rather abrupt rise in the amino-acid nitrogen (Chart 1). After a smaller dose of toxin, i.e., one causing death in from 4 to 7 days, the changes are slower in appearing, but just as definite (Chart 2). These are a gradual, but progressive rise in the nonprotein nitrogen, a tendency for the blood

sugar either to change little or to rise to definite hyperglycemic levels, and a slight to moderate rise in the amino acid nitrogen early with in some cases a tendency for the amino acid nitrogen to decrease subsequently, or remain moderately elevated until the death of the animal

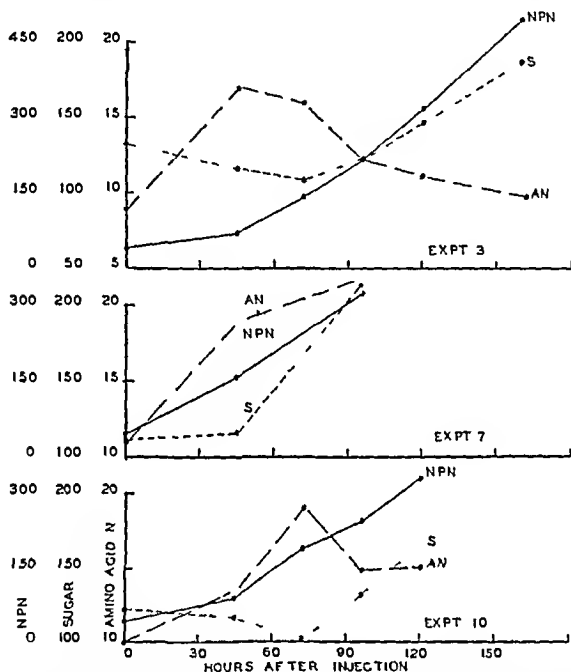


CHART 2 CHANGES OF BLOOD SUGAR, NONPROTEIN NITROGEN AND AMINO ACID NITROGEN FOLLOWING THE INJECTION OF 0.8 MINIMAL LETHAL DOSE OF DIPHTHERIA TOXIN

The nonprotein nitrogen rises at a regular rate with both doses of toxin. Although the nonprotein nitrogen increases more rapidly in the animals receiving the larger dose of toxin, it attains greater final concentration in the animals receiving the smaller dose of toxin. Apparently the final height of the nonprotein nitrogen is dependent chiefly on the duration of the intoxication.

The sugar and amino acid nitrogen values present an interesting relationship. This can be more clearly observed in some of the individual experiments. With the larger dose of toxin, as the blood sugar fell, the

amino-acid nitrogen rose For example, in Experiment 11 (see Chart 1), the blood sugar after 24 hours was 113 mgm per cent and the amino-acid nitrogen 35 mgm per cent Coincident with the fall in sugar to 65 the amino-acid nitrogen rose to 55 Again, in Experiment 15, as the sugar fell from 117 to 73 the amino-acid nitrogen rose from 16.1 to 41.5 Similarly, in Experiment 13, the amino-acid nitrogen rose from 15 to 60 while the sugar fell from 135 to 108 In general it may be said that in the rabbits that die before the 3d day after having received relatively large doses of diphtheria toxin, the hypoglycemia is almost invariably accompanied by a marked amino-acidemia

In the experiments in which the rabbits received smaller doses of toxin and survived from 4 to 7 days, the situation was somewhat different The changes can best be pointed out in the experiments in which the animals survived the longest, namely, Experiments 3 and 10 (Chart 2) In these there is, at first, a very slight fall in the blood sugar values, coincident with a slight rise in the amino-acid nitrogen Later, as the hyperglycemia develops, there is a tendency for the amino-acid to remain relatively stationary or to return to the pre-injection level As has been pointed out, not all of the animals receiving the small dose of toxin develop hyperglycemia, nor do all show this relationship between the height of the blood sugar and the amino-acidemia as definitely as it has been described above The significance of this will be discussed later

PATHOLOGY

A number of histological sections were made of the various organs of the animals used in the chemical studies Although these sections do not demonstrate any facts not already recognized, they permit correlation of the anatomical changes with the various stages of diphtheritic intoxication manifested in the chemical disturbances Briefly, the histological changes in the animals receiving the larger dose of toxin were as follows (1) Necrosis of the liver parenchyma involving in some cases the major part of the liver cells (2) A tubular and glomerular nephritis which in extreme cases led to necrosis of large areas of the kidneys (3) Degeneration of heart muscle fibers (4) Hemorrhages in various organs including the adrenals and intestines The last finding was not constant, but occurred in the animals most severely affected The findings in the rabbits receiving the smaller doses were similar but less acute and extensive The myocardial fibers were occasionally surrounded by round cells and some repair was apparently taking place The liver in these, also, showed necrosis, which in some cases was limited to the area surrounding the central vein, with dilatation of the liver capillaries which gave a picture indistinguishable from that of chronic passive congestion The kidney lesions were definite but did not seem to be necessarily irreparable Lesions of the adrenal parenchyma, except in the presence of hemorrhage, could not be recognized with certainty after either dose All the animals showed a

certain degree of dehydration as evidenced by diminished skin turgor, a relative increase in the concentration of hemoglobin (30) and loss of weight of 10 to 20 per cent in typical cases. All these findings have been described in human cases. We would particularly call attention to the liver and kidney lesions which have been overlooked most frequently.

DISCUSSION

The elevation of the nonprotein nitrogen can be most logically related to nephritis. This is confirmed also by the fact that the elevation of amino acid nitrogen does not parallel that of the nonprotein nitrogen. Undoubtedly dehydration aggravated the renal insufficiency. Control experiments demonstrated that starvation alone may lead to an elevation of the nonprotein nitrogen but this rarely exceeded 75 mgm per cent.

Previous work indicates that liver injury is the chief cause of elevation of amino acid nitrogen. Marshall and Rowntree (24) found elevated blood amino acid nitrogen in the terminal stages of phosphorus and chloroform poisoning. Lewis and Izume (25) found a similar elevation in hydrazine poisoning in rabbits. Elevations of amino acid nitrogen are also reported in acute yellow atrophy (Rabinowitch (26)), and in experimental yellow fever (Wakeman and Morrell (27)). Apparently the margin of safety in the deamination function of the liver is so great that only extreme destruction of the liver leads to increase in the amino acid nitrogen. Although an increase has been reported in rare cases of uremia, nephritis is not usually considered a cause of amino acidemia. The degree to which the amino-acid nitrogen increased in the rabbits given diphtheria toxin is greater than that reported in severe hepatic disease in humans, except in a few instances (Rabinowitch). The data demonstrate marked disturbances of liver function corresponding to the extensive liver necrosis.

We have been unable to find reports of blood amino acid nitrogen determinations in patients with diphtheria and have only had opportunity to make this determination ourselves in two cases of severe diphtheria. In neither was there an elevation of amino acid nitrogen although one patient developed definite myocardial degeneration and severe neuritis. Probably only a small number of the most severe cases will show an elevation of the amino acid nitrogen.

With the recognition of the role of hepatic degeneration in severe diphtheria intoxication, the abnormality of carbohydrate metabolism receives an adequate explanation. With the greatest degrees of liver injury, the function of deamination is diminished and presumably other related processes which serve to convert non carbohydrate material to glucose fail. The loss of stored glycogen and the breakdown of the processes of glycconeogenesis lead to hypoglycemia. In Paper II of this series (29a) failure of the liver injured by diphtheria toxin to store glycogen is demonstrated. These findings are in agreement with the work of Corkill (28). This inability to store glycogen occurs in the animals receiving large and

small doses of toxin and can be demonstrated in animals at stages of the intoxication when no elevation of amino-acid can be demonstrated. With persistence of hepatic glycconeogenesis and impairment of hepatic glycconeogenesis, hyperglycemia develops. Since varying degrees of failure of these two hepatic functions may occur simultaneously, varying degrees of hypo- and hyperglycemia are found. As has been pointed out, hypoglycemia seems to be related to the rapidity of the increase of amino-acid nitrogen, and hyperglycemia to the period of survival when no increase in the amino-acid nitrogen occurs.

The relation of failure of hepatic glycconeogenesis to accumulation of blood lactic acid is presumably close. An attempt was made to study this by daily blood lactic acid determinations. The data are presented in Tables I and II. One hesitates to draw any conclusions from this material because

TABLE I

Changes in blood sugar, lactic acid and nonprotein and amino acid nitrogen following the intravenous injection of 3 minimal lethal doses of diphtheria toxin

Experiment number	Period following injection of toxin	Nonprotein nitrogen	Sugar	Amino acid nitrogen	Lactic acid	Period of survival
	hours	mgm per cent	mgm per cent	mgm per cent	m Eq per liter	hours
11		50	103	7.0	2.4	50
	24	206	113	37.0	8.4	
	48	250	65	65.0	12.7	
12	24	40 170	135 77	7.5 37.0	4.3 14.8	28
13		50	129	10.2	3.4	59
	24	100	135	15.0	6.6	
	48	225	118	41.0	8.1	
	54	265	108	60.0	9.0	
14	24	44 90	133 72	11.0 27.5	7.6 9.7	34
15		56	128	10.8	5.7	40
	24	100	117	16.1	4.9	
	36	220	73	41.5	5.8	
16	24	40 92	116 63	11.5 32.0	6.1 7.2	27
17	24	45 100	119 82	10.2 27.8	6.9 10.3	32
18	24	50 133	128 101	9.0 29.5	5.0 7.2	36
19		48	117	9.4	6.3	63
	36	200	128	34.5	4.8	
	60	350	106	66.0	12.0	

TABLE II

Changes in blood sugar lactic acid and nonprotein and amino acid nitrogen following the intravenous injection of $\frac{3}{4}$ minimal lethal dose of diphtheria toxin

Experiment number	Period following injection of toxin	Nonprotein nitrogen	Sugar	Amino acid nitrogen	Lactic acid	Period of survival
	hours	mgm per cent	mgm per cent	mgm per cent	m Eq per liter	hours
1	48	40	123	10.3	9.7	128
	96	148	122	15.0	6.2	
	120	320	150	14.6	5.8	
		410	158	11.4	6.8	
2	48	43	136	9.5	10.7	176
	96	80	112	13.2	6.2	
	120	125	137	14.0	8.2	
	144	210	218	14.9	7.8	
	172	260	196	12.5	5.0	
		380		12.8	5.0	
3	48	40	133	9.4	9.3	184
	72	71	116	17.3	8.2	
	96	144	109	16.6	5.6	
	120	215	122	12.3	4.8	
	168	320	149	11.6	5.6	
		500	187	10.0	7.7	
4	48	44	105	8.8	7.1	97
	72	91	123	15.8	6.3	
	96	240	129	14.0	7.5	
		450	109	16.9	6.5	
5	48	42	127	9.7	8.6	90
	72	100	118	14.4	7.3	
		380	160	16.0	5.0	
6	48	33	119	8.8	9.2	104
	96	105	118	15.4	8.7	
		350	122	21.0	12.7	
7	48	45	111	11.0	4.8	104
	96	154	114	19.5	10.5	
		330	215	22.0	9.2	
8	48	44	131	9.8	9.5	82
	72	85	120	13.9	8.2	
		230	132	34.0		
9	48	48	140	9.5	6.0	98
	72	73	116	13.7	6.4	
	96	185	134	27.0		
		300	237	32.0		
10	48	40	122	9.9	5.6	128
	72	83	116	13.4	12.5	
	96	190	100	19.0		
	120	250	132	14.8		
		340	165	15.2		

of the wide variation in blood lactic acid levels in the normal animals. The pre-injection levels were so frequently markedly elevated, due to struggling during the removal of blood samples from the hearts, that we regard lactic acid determinations in rabbits as unreliable indices of the state of blood lactic acid under resting conditions. In general, one may say that with the less severe intoxication there is less tendency for the lactic acid to reach the markedly elevated levels that are sometimes encountered in the severely intoxicated rabbits.

A similar unreliability of the blood sugar values might be suspected. However, essentially the same concentrations as those reported in this paper were found in blood taken from the ear when struggling was minimal. This fact, together with uniformity of the trend of the results, leads us to consider the blood sugar values reliable.

SUMMARY

1 The blood sugar, amino-acid nitrogen, nonprotein nitrogen, and lactic acid were studied in fasting rabbits which had intravenous injections of small and large doses of diphtheria toxin.

2 During the severe intoxication, hypoglycemia, marked amino-acidemia and azotemia were found.

3 A tendency was demonstrated for the hypoglycemia to occur while the amino-acid nitrogen was rapidly increasing.

4 During the mild intoxication hyperglycemia could be demonstrated in most of the animals that survived for 5 or more days. Under these circumstances the amino-acid nitrogen was only slightly elevated. Non-protein nitrogen became markedly elevated.

5 It is pointed out that the azotemia is probably related to nephritis produced by the toxin and perhaps aggravated by dehydration. The amino-acidemia is apparently brought about by extensive liver necrosis. The varying degrees of hypo- and hyperglycemia probably are related to varying degrees of failure of hepatic glycconeogenesis and glycogenesis.

BIBLIOGRAPHY

- 1 Rosenthal, F, *Arch f exper Path u Pharmakol*, 1914, lxy, 99. Über die Störungen des Kohlenhydratstoffwechsels bei der experimentellen Diphtherievergiftung.
- 2 Tisdall, F F, Drake, T G H, and Brown, A, *Am J Dis Child*, 1926, xxxii, 854. The Production of a Lowered Carbohydrate Tolerance in Dogs.
- 3 Sweeney, J S, and Lackey, R W, *Arch Int Med*, 1928, xli, 257. The Effect of Toxemia on Tolerance for Dextrose.
- 4 Sweeney, J S, *Arch Int Med*, 1928, xli, 420. Effect of Toxemia on Tolerance for Dextrose and on the Action of Insulin.
- 5 Mikami, S *Tohoku J Exper Med*, 1925, vi, 299. The Blood Sugar Level and the Epinephrin Content of the Suprarenals of the Rabbit in Diphtheritic Intoxication.

- 6 Schwentker, F F and Noel, W W, Bull Johns Hopkins Hosp 1930, xlv, 259 The Circulatory Failure of Diphtheria II The Carbohydrate Metabolism in Diphtheria Intoxication
Ibid 1930, xlv, 359 The Circulatory Failure of Diphtheria III The Treatment of the Circulatory Failure of Diphtheria
- 7 Lawrence R D, and Buckley O B Brit J Exper Path, 1927 viii, 58 The Inhibition of Insulin Action by Toxemias and its Explanation I The Effect of Diphtheria Toxin on Blood Sugar and Insulin Action in Rabbits
- 8 Netzeley, R E, Am J Dis Child, 1929, xxxvii 511 The Effect of Diphtheria Toxin on the Action of Insulin, a Study of the Effect of Infections on Carbohydrate Metabolism in Diabetes Mellitus
- 9 Hector, F J, Lancet, 1926, ii, 642 Carbohydrate Metabolism in Diphtheria
- 10 Elkeles, A, and Hemmann, F, Deutsches Arch f klin Med, 1927, clv 263 Über Störungen im Kohlenhydratstoffwechsel bei Diphtherie.
Ibid, 1928, clviii 238 Weitere Untersuchungen über Störungen im Kohlenhydratstoffwechsel bei Diphtherie
- 11 Prochajka M Y Bull et mem Soc med d hop de Paris, 1930, liv, 865 Recherches sur la glycémie et le taux de l'azote non protéique dans les diphthéries graves
- 12 Benn E C, Hughes, E, and Alstead S, Lancet 1932 i 281 Toxic Diphtheria Combined Antitoxin and Dextrose insulin Therapy
- 13 Brems A, Ugesk f laeger 1932 xciv, 292 Blodsukkerstudier hos Difterteripatienter
- 14 Hibbard, C M, and Morrissey, M J, J Exper Med 1899, iv, 137 Glycosuria in Diphtheria
- 15 Karsner, H T, and Deuss, W, J Exper Med 1914, xix, 259 A Further Study of Nitrogen Retention in the Blood in Experimental Acute Nephritis
- 16 Glesinger Reischer G, and Glesinger, B, Wien klin Wchnschr 1923, xxxvi, 181 Ueber die Reststickstoffwerte bei Diphtherie und Beeinflussung derselben durch Diphtherieheilserum
- 17 Lereboullet P, Gournay J J and Donato, J Ann de med 1931 xxxix 547 La fonction renale dans la diphthérie Azotémie et diphthérie
- 18 Hanner J P, and Whipple, G H, Arch Int Med 1931, xliiii, 598 The Elimination of Phenolsulphonphthalein by the Kidney, the Influence of Pathologic Changes in the Liver
- 19 Derot, M La créatininémie Vigne Paris, 1932
- 20 Peters J P and Van Slyke, D D, Quantitative Clinical Chemistry II Methods p 465 Williams and Wilkins Co Baltimore, 1932
- 21 Ibid, page 427
- 22 Ibid, page 584
- 23 Schmidt E G Arch Int Med, 1929, xlv, 351 The Amino acid Content of the Blood in Health and in Disease
- 24 Marshall E K, Jr, and Rowntree, L G J Exper Med 1915 xxii 333 Studies in Liver and Kidney Function in Experimental Phosphorous and Chloroform Poisoning
- 25 Lewis, H B, and Izume S, J Biol Chem, 1926 lxxi, 33 The Influence of Hydrazine and its Derivatives on Metabolism II Changes in the Non protein Nitrogenous Constituents of the Blood and in the Metabolism of Injected Glycine in Hydrazine Intoxication

- 26 Rabinowitch, I M, J Biol Chem, 1929, lxxiii, 333 Biochemical Findings in a Rare Case of Acute Yellow Atrophy of the Liver, with Particular Reference to the Origin of Urea in the Body
- 27 Wakeman, A M, and Morrell, C A, Arch Int Med, 1930, xlv, 290 Chemistry and Metabolism in Experimental Yellow Fever in Macacus Rhesus Monkeys I Concentration of Non-protein Nitrogenous Constituents in the Blood
- 28 Corkill, B, J Physiol, 1932, lxxv, 381 The Influence of Toxemia on Carbohydrate Metabolism
- 29a Yannet, H, and Darrow, D C, J Clin Invest, 1933, xi, 779 Physiological Disturbances during Experimental Diphtheritic Intoxication II Hepatic Glycogenesis and Glycogen Concentration of Cardiac and Skeletal Muscle
- b Yannet, H, and Goldfarb, W, J Clin Invest, 1933, xi, 787 Physiological Disturbances during Experimental Diphtheritic Intoxication III Respiratory Quotients and Metabolic Rate
- 30 Darrow, D C, Yannet, H, and Cary, M Katharine, (To be published), Physiological Disturbances during Experimental Diphtheritic Intoxication IV Blood Electrolyte and Hemoglobin Concentrations

PHYSIOLOGICAL DISTURBANCES DURING EXPERIMENTAL DIPHThERITIC INTOXICATION II HEPATIC GLYCO- GENESIS AND GLYCOGEN CONCENTRATION OF CARDIAC AND SKELETAL MUSCLE¹

By HFRMAN YANNET AND DANIEL C. DARROW

*(From the Department of Pediatrics Yale University School of Medicine
New Haven)*

(Received for publication May 20 1933)

In the first paper of this series (12) it was pointed out that during diphtheritic intoxication in rabbits there was evidence of liver disturbance, manifested by a marked rise in the amino acid nitrogen which in the more severe intoxications was accompanied by a hypoglycemia. When smaller doses of toxin were injected the amino acid nitrogen changed but little, while the blood sugar remained relatively normal or rose to hyperglycemic levels. Accompanying these changes the nonprotein nitrogen rose independently of the amino acid nitrogen, a finding which seemed to be associated with the nephritis which could be demonstrated histologically.

In order to associate, if possible, these findings of hypoglycemia, hyperglycemia and normal sugar values with liver injury, the assumption was made that the two chief functions of the liver concerned with carbohydrate metabolism may be injured separately or in varying degrees. It was felt that the failure of deamination as indicated by the elevated amino-acid nitrogen concentrations could be interpreted as a type of injury that might be associated with the failure of hepatic glycogenesis leading to hypoglycemia. However if the formation of glucose from non carbohydrate material is only slightly impaired, a failure of hepatic glycogenesis would result in hyperglycemia.

The following experiments were designed to study the ability of rabbits suffering from diphtheritic intoxication to store glycogen in the various tissues. The effect of intravenous injections of glucose with and without insulin, on the liver and heart glycogen concentrations, is reported.

PROCEDURE AND METHODS

Rabbits weighing about 1500 grams were injected intravenously with 3/4 to 1 minimal lethal dose of diphtheritic toxin. This dose caused death in from 3 to 7 days. The rabbits were on a stock diet consisting of oats and greens until 24 hours before the injection of the toxin when the food was removed.

¹ This work was aided by a grant from The Corn Products Refining Company.

from the cages. Starvation was maintained throughout the balance of the experiment, water was offered freely. The level of the blood nonprotein nitrogen was used as a fairly reliable index of the severity of the intoxication, this was determined daily and when it reached 100 to 150 mgm per cent or higher the operative phase of the experiment was started. (Previous experience indicated that diphtheritic rabbits with a blood nonprotein nitrogen level of this magnitude die within 24 to 48 hours.) As an anesthetic, one-half cubic centimeter of a 10 per cent freshly prepared amytal solution per kilogram of body weight was injected intraperitoneally. Following the development of adequate anesthesia the liver was exposed by a mid-line upper abdominal incision. A section of liver weighing approximately 3 to 5 grams was removed, bleeding being effectively controlled by a ligature, and was immediately cut into small pieces and dropped into a weighed tube containing 60 per cent potassium hydroxide for glycogen determination. In one series of animals 5 cc of a 50 per cent glucose solution was then injected intravenously immediately, and again after an interval of 20 minutes. In another series the same technique was employed except that 2 1/2 units of insulin were injected along with the glucose solution. One hour following the last administration of glucose another section of liver was obtained for glycogen determination. (Preliminary experiments had shown that the increase of liver glycogen was approximately the same after one as it was after two hours.) The hearts were removed at the same time as the second liver section. The controls were subjected to precisely the same procedure, including the starvation regime, for a similar period of time.

Data referable to the glycogen content of liver, heart and skeletal muscle were obtained from another series of animals on a similar fasting regime. The tissues were removed within 30 seconds after the animal was stunned by a blow at the base of the skull. At the time of these determinations the blood nonprotein nitrogen of the diphtheritic animals was about 150 to 200 mgm per cent. The same dose of toxin was used in this series as in the one previously described.

The glycogen method employed was fashioned after Pfluger's original procedure but was sufficiently modified to warrant description. About 5 grams of tissue were cut into small sections as soon as possible after removal from the animal and collected in a weighed centrifuge tube of 100 cc capacity containing 10 cc of 60 per cent potassium hydroxide solution, which was then reweighed. Digestion was allowed to take place in a steam bath for a period of three hours, or until a relatively clear liquid resulted. Approximately 75 to 80 cc of 95 per cent alcohol were then added and the tube allowed to remain in a refrigerator overnight to insure complete glycogen precipitation. The tube was then centrifuged at moderate speed for 10 minutes, the supernatant fluid siphoned off and the glycogen precipitate washed with 25 cc of 95 per cent alcohol. Following another centrifugation for 10 minutes the supernatant fluid was again removed. One drop of phenolphthalein and 25 cc of water were added and the resulting solution neutralized by the careful addition of 25 per cent hydrochloric acid. (This usually required 4 to 5 drops.) Two and one-half cc of the acid were then added, producing a final acidity approximately equivalent to a 2 1/2 per cent hydrochloric acid solution. The glycogen was then hydrolyzed by heating in a steam bath for three hours. The resulting solution was neutralized and made up to a suitable volume and the glucose content determined in aliquots by Somogyi's modification of the Shaffer-Hartmann sugar method. The glycogen content is expressed as milligrams of glucose per gram of tissue. The method has an error in duplicates of about 8 per cent.

RESULTS

In Chart 1 are graphically tabulated the results of the determinations of the glycogen content of liver, muscle and heart, in both diphtheritic and normal animals. There are two striking observations to be made: first, that there is essentially no difference in the glycogen content of the heart and skeletal muscles of the diphtheritic as compared to the normal animals; second, that there is a definite diminution in the glycogen content of diphtheritic livers as compared to normals.

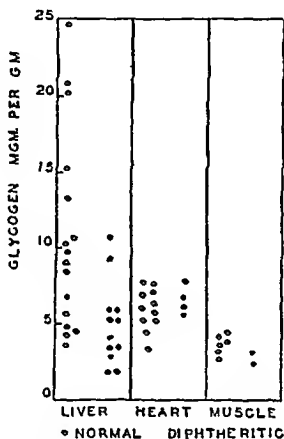


CHART 1 THE GLYCOGEN CONCENTRATION OF LIVER HEART AND MUSCLE IN NORMAL AND DIPHTHERITIC RABBITS

The glycogen content of normal and diphtheritic livers before and after the injection of glucose and glucose plus insulin is graphically represented in Chart 2. The glycogen concentration of the liver before and after the glucose and glucose plus insulin injections is represented by a circle and arrow end respectively. The length of the intervening line represents the actual increase in glycogen concentration in milligrams per gram of tissue. In the normal animals the injection of glucose brought about an increase in the glycogen content of the liver which varied from 6 to 29 mgm per gram of tissue, in other words an increase of from approximately 0.5 to 2 per cent in glycogen concentration. The addition of insulin, it will be seen, had no appreciable effect in normal animals a finding which is similar to that described by other investigators.

In the diphtheritic animals on the other hand, following the injection of glucose the increase in liver glycogen varied from less than 1 mgm to

In Chart 3 the results of the glycogen determinations on the hearts of the normal and diphtheritic animals, following the intravenous injection of glucose, with and without insulin, are represented. These have been compared to the values found in the untreated animals. There is no significant difference in the cardiac glycogen of normal and diphtheritic animals following injections of glucose. However, the cardiac glycogen of diphtheritic animals following the injection of glucose plus insulin may be significantly greater than in the other groups.

DISCUSSION

Early in this investigation it was appreciated that diphtheritic rabbits refuse all food relatively soon after receiving an injection of toxin and should, therefore, be regarded as fasting animals. Since one of the striking effects of starvation is a depletion of the glycogen stores, it was apparent that in a comparative study of glycogen content of various tissues in normal and intoxicated animals, one would have to consider not only the effect of the diphtheria toxin but also of starvation. It was felt that the most practical procedure for minimizing this difficulty was to maintain both groups of animals on a similar fasting regime. We thus felt that any differences encountered in the glycogen concentrations of the examined tissues could, with greater reliability, be attributed to the effect of the diphtheria toxin alone.

Although diphtheria both clinically and experimentally, is a generalized disease process and not limited to any one particular organ, it is nevertheless true that the myocardial involvement is an outstanding and relatively frequent manifestation of a severe intoxication. Warthin (1) in his review of the anatomical changes in the heart in diphtheria suggested the possibility that some of the encountered lesions may well be related to a nutritional disturbance. Schwentker and Noel (2), who, on the basis of their experimental and clinical investigations and a review of the literature, stressed the theory of insulin dysfunction as the cause of the carbohydrate disturbance, believed that failure of glycogenesis played an important role in the myocardial insufficiency. It was therefore with interest that we noted no essential difference in the glycogen concentration of the hearts of diphtheritic and normal animals. As far as we can determine from a review of the available literature there have been no determinations of heart glycogen in diphtheritic animals.

The few determinations of heart glycogen before and after injection of glucose cannot be interpreted with certainty. There is no evidence that the amount of glucose injected increased the heart glycogen in either the diphtheritic or normal animals. The group of diphtheritic animals subjected to intravenous injection of glucose plus insulin have slightly higher heart glycogens than the controls. The difference in the averages is just three times the probable error of the differences. However, in a small

number of determinations such a difference cannot be regarded as more than suggestive

An occasional reference to muscle glycogen determinations in experimental diphtheritic intoxication is found in the literature. Schwenker and Noel found the muscles of three animals completely devoid of glycogen and in a fourth, 0.16 per cent. Rosenthal (3) in three rabbits found the muscle glycogen to be 0.165, 0.245 and 0.305 per cent respectively. We have found in six animals the glycogen concentration to vary from 0.23 to 0.43 per cent, but we have also found that in six normal animals under a similar starvation régime the muscle glycogen was approximately of similar magnitude, varying from 0.26 to 0.42 per cent. In other words, as in the case of cardiac glycogen, no essential difference could be demonstrated between the muscle glycogen of normal and diphtheritic animals.

An entirely different picture is presented when one examines the values for the glycogen concentration of the livers in the diphtheritic animals. In this case there is a definite decrease in the intoxicated rabbits, the glycogen content varying from 0.12 to 0.63 per cent, and averaging about 0.35 per cent, while in the normal but fasted rabbits the concentration in the liver varies from 0.4 to 2.0, averaging about 1.0 per cent. Whether the liver shares to a greater extent in the general systemic intoxication than does either the heart or muscle, as these data would seem to imply, is not absolutely clear. Histologically the liver exhibits more injury than the cardiac or skeletal muscle. However, it is probable that a large part of the liver glycogen serves primarily as storage material, and that it is the diminution in this fraction which accounts for the marked difference in the glycogen concentration encountered. On the other hand in the heart and to a lesser extent in muscle, most of the glycogen content is intimately related to the immediate metabolic needs of the tissues in question, and is, therefore, less likely to be involved in an injury of relatively similar degree than is the glycogen of the liver.

The data obtained in the study of hepatic glycogenesis show a marked disturbance in the ability of the diphtheritic liver either to store or to form glycogen or both. Furthermore, the injection of insulin does not correct this deficiency. Numerous investigators have demonstrated a marked decrease in carbohydrate tolerance as evidenced by diabetic-like sugar tolerance curves. Moreover, Lawrence and Buckley (4), Netzley (5) and Sweeney (6) in animals, and Elkeles and Hermann (7) and Schwenker and Noel (2) in human cases of diphtheria, have found a decided impairment in the normal depressing action of insulin on the sugar tolerance curves. As was previously pointed out, this has been interpreted by some to signify insulin dysfunction or actual insufficiency. It is apparent that an inability of the liver to remove glucose for storage as glycogen would adequately explain a delayed or elevated sugar tolerance curve and apparent refractoriness to insulin. Obviously, such a failure of hepatic glycogenesis

does not necessarily signify any inability to burn carbohydrate. In the third paper of this series we demonstrate by means of respiratory quotients determined during the course of the intoxication that diphtheritic rabbits can burn carbohydrate.

The occurrence of severe liver injury during diphtheritic intoxication is by no means unrecognized. Pathologically this was noted many years ago by Babes (8), Welch and Flexner (9), Fahr (10), and others. Histologically, the lesion consists essentially of varying degrees of necrosis in the region of the central vein. From our own experience in examining numerous sections of liver obtained from diphtheritic animals, this has been the most constant and striking pathological finding. In many cases the destruction was so extensive that very little normal hepatic tissue could be seen.

It may be pertinent, in this connection, to point out the striking similarity which exists, as far as evidence of disturbance in carbohydrate metabolism is concerned between diphtheritic intoxication and other conditions in which liver injury is the principal pathological finding, namely, phosphorus, chloroform and hydrazine poisoning and yellow fever. In these conditions there have been demonstrated hypoglycemia, diabetic-like sugar tolerance curves, marked reduction in liver glycogen and increases in blood amino-acid nitrogen (11).

SUMMARY

1 The liver glycogen of fasted rabbits injected intravenously with diphtheria toxin is definitely diminished in comparison with that of controls subjected to similar periods of fasting. Under these conditions no significant difference was noted in the concentration of glycogen in heart and muscle of diphtheritic and control rabbits.

2 A marked diminution in hepatic glycogenesis following the intravenous injection of glucose was noted in diphtheritic rabbits. The injection of insulin along with the glucose did not alter hepatic glycogenesis.

3 The concentrations of heart glycogen of normal and diphtheritic rabbits following the injection of glucose with and without insulin, showed no significant differences.

4 It is pointed out that the destruction of liver parenchyma demonstrated anatomically in diphtheritic intoxication is accompanied by a failure of hepatic glycogenesis which is responsible for some of the disturbances in carbohydrate metabolism described in this condition.

BIBLIOGRAPHY

- 1 Warthin A S, *J Infect Dis* 1924 **xxv**, 32. The Myocardial Lesions of Diphtheria.
- 2 Schwentker F F and Noel W W. *Bull Johns Hopkins Hosp* 1930 **xlvi**, 259. The Circulatory Failure of Diphtheria. II. The Carbohydrate Metabolism in Diphtheria Intoxication.

number of determinations such a difference cannot be regarded as more than suggestive

An occasional reference to muscle glycogen determinations in experimental diphtheritic intoxication is found in the literature. Schwentker and Noel found the muscles of three animals completely devoid of glycogen and in a fourth, 0.16 per cent. Rosenthal (3) in three rabbits found the muscle glycogen to be 0.165, 0.245 and 0.305 per cent respectively. We have found in six animals the glycogen concentration to vary from 0.23 to 0.43 per cent, but we have also found that in six normal animals under a similar starvation regime the muscle glycogen was approximately of similar magnitude, varying from 0.26 to 0.42 per cent. In other words, as in the case of cardiac glycogen, no essential difference could be demonstrated between the muscle glycogen of normal and diphtheritic animals.

An entirely different picture is presented when one examines the values for the glycogen concentration of the livers in the diphtheritic animals. In this case there is a definite decrease in the intoxicated rabbits, the glycogen content varying from 0.12 to 0.63 per cent, and averaging about 0.35 per cent, while in the normal but fasted rabbits the concentration in the liver varies from 0.4 to 2.0, averaging about 1.0 per cent. Whether the liver shares to a greater extent in the general systemic intoxication than does either the heart or muscle, as these data would seem to imply, is not absolutely clear. Histologically the liver exhibits more injury than the cardiac or skeletal muscle. However, it is probable that a large part of the liver glycogen serves primarily as storage material, and that it is the diminution in this fraction which accounts for the marked difference in the glycogen concentration encountered. On the other hand in the heart and to a lesser extent in muscle, most of the glycogen content is intimately related to the immediate metabolic needs of the tissues in question, and is, therefore, less likely to be involved in an injury of relatively similar degree than is the glycogen of the liver.

The data obtained in the study of hepatic glycogenesis show a marked disturbance in the ability of the diphtheritic liver either to store or to form glycogen or both. Furthermore, the injection of insulin does not correct this deficiency. Numerous investigators have demonstrated a marked decrease in carbohydrate tolerance as evidenced by diabetic-like sugar tolerance curves. Moreover, Lawrence and Buckley (4), Netzley (5) and Sweeney (6) in animals, and Elkeles and Heimann (7) and Schwentker and Noel (2) in human cases of diphtheria, have found a decided impairment in the normal depressing action of insulin on the sugar tolerance curves. As was previously pointed out, this has been interpreted by some to signify insulin dysfunction or actual insufficiency. It is apparent that an inability of the liver to remove glucose for storage as glycogen would adequately explain a delayed or elevated sugar tolerance curve and apparent refractoriness to insulin. Obviously, such a failure of hepatic glycogenesis

PHYSIOLOGICAL DISTURBANCES DURING EXPERIMENTAL DIPHTHERITIC INTOXICATION III RESPIRATORY QUOTIENTS AND METABOLIC RATE¹

BY HERMAN YANNET AND WALTER GOLDFARB

(From the Department of Pediatrics and Physiology Yale University School of
Medicine New Haven)

(Received for publication May 20 1933)

Previous investigators have demonstrated disturbances of carbohydrate metabolism during both clinical and experimental diphtheritic intoxication. These disturbances are made manifest by delayed sugar tolerance curves, high blood sugar values, and, occasionally, glycosuria. These observations have led to the assumption on the part of many that, during the course of diphtheria, there exists a relative insulin deficiency or dysfunction, a detailed review of this phase of the literature is given in Paper I of this series (1). In the two preceding papers of this series (1, 7) we have demonstrated by histological and chemical studies, a widespread destruction of liver parenchyma with derangement of liver function following the intravenous injection of diphtheria toxin in rabbits. Physiologically, this manifested itself by varying degrees of disturbance in hepatic glycconeogenesis and hepatic glycogenesis. The belief was expressed that the above mentioned disturbances in carbohydrate metabolism could be related to the hepatic injury. In the following communication are reported the results of a study of the respiratory quotients in rabbits, aimed to determine what effect, if any, diphtheritic intoxication has upon their ability to burn carbohydrates.

METHOD

The apparatus used for the determination of respiratory quotients was described by Marine (2) in 1922 and is based on the Haldane open-circuit system. It was adequately tested before the experiments were begun for proper absorption of carbon dioxide and water and for air tight connections.

Rabbits in good condition, and weighing from 900 to 1400 grams were employed for the investigation. Both control and diphtheritic animals were deprived of food 24 hours before the onset of the experiment. Starvation was maintained throughout the balance of the experiment only water being offered. The diphtheria toxin used was of a strength such that 0.016 cc. was equivalent to 1 minimal lethal dose of toxin. Three quarters to 1 minimal lethal dose was injected intravenously. It was previously found that this dose would bring about the death of the fasted animals in from three to seven days. The animals

¹ This work was aided by a grant from The Corn Products Refining Company.

- Ibid, 1930, xvi, 359 The Circulatory Failure of Diphtheria III The Treatment of the Circulatory Failure of Diphtheria
- 3 Rosenthal, F, Arch f exper Path u Pharmacol, 1914, lxxv, 99 Über die Störungen des Kohlenhydratstoffwechsels bei der experimentellen Diphtherievergiftung
 - 4 Lawrence, R D, and Buckley, O B, Brit J Exper Path, 1927, viii, 58 The Inhibition of Insulin Action by Toxemias and its Explanation I The Effect of Diphtheria Toxin on Blood Sugar and Insulin Action in Rabbits
 - 5 Netzeley, R E, Am J Dis Child, 1929, xxxvii, 511 The Effect of Diphtheria Toxin on the Action of Insulin, a Study of the Effect of Infections on Carbohydrate Metabolism in Diabetes Mellitus
 - 6 Sweeney, J S, Arch Int Med, 1928, xli, 420 Effect of Toxemia on Tolerance for Dextrose and on the Action of Insulin
 - 7 Elkeles, A, and Heilmann, F, Deutsches Arch f klin Med, 1927, clv, 263 Über Störungen im Kohlenhydratstoffwechsel bei diphtherie
Ibid, 1928, clviii, 238 Weitere Untersuchungen über Störungen im Kohlenhydratstoffwechsel bei Diphtherie
 - 8 Babes, V, Virchows Arch f path Anat, 1890, cix, 460 Untersuchungen über den Diphtheriebacillus und die experimentelle Diphtherie
 - 9 Welch, W H, and Flexner, S, Bull Johns Hopkins Hosp, 1891, ii, 107 The Histological Changes in Experimental Diphtheria
 - 10 Fahr, T, Virchows Arch f path Anat, 1916, ccxxi, 38 Beiträge zur Diphtherie-Frage
 - 11 A Bodansky, M, J Biol Chem, 1923, lviii, §15 The Effect of Chloroform and Phosphorous Poisoning on Carbohydrate Tolerance
B Ibid, Am J Physiol, 1923, lxxvi, 375 The Production of Hypoglycemia in Experimental Derangements of the Liver
C Hendrix, B M, and McAmis, A J, J Biol Chem, 1924, lxi, 45 Alkalosis Produced by Injections of Hydrazine Sulfate in Dogs
D Lewis, H B, and Izume, S, J Biol Chem, 1926, lxxi, 33 The Influence of Hydrazine and its Derivatives on Metabolism II Changes in the Non-protein Nitrogenous Constituents of the Blood and in the Metabolism of Injected Glycine in Hydrazine Intoxication
E Ibid, 1926, lxxxi, 51 III The Mechanism of Hydrazine Hypoglycemia
F Williamson, C S, and Mann, F C, Am J Physiol, 1923, lxxv, 267 Studies on the Physiology of the Liver V The Hepatic Factor in Chloroform and Phosphorous Poisoning
G Underhill, F P, and Hogan, A G, J Biol Chem, 1914, xx, 203 Studies in Carbohydrate Metabolism VIII The Influence of Hydrazine on the Utilization of Dextrose
H Wakeman, A M, and Morrell, C A, Arch Int Med, 1931, xlvii, 104 Chemistry and Metabolism in Experimental Yellow Fever in Macacus Rhesus Monkeys III Blood Sugar and Liver Glycogen
I Ibid, 1931, xlviii, 301 Chemistry and Metabolism in Experimental Yellow Fever in Macacus Rhesus Monkeys IV Tolerance Tests for Dextrose
J Ibid, 1932, xlix, 826 Chemistry and Metabolism in Experimental Yellow Fever in Macacus Rhesus Monkeys V Acid-base and Electrolyte Equilibrium
 - 12 Yannet, H, and Darrow, D C, J Clin Invest, 1933, xii, 767 Physiological Disturbances during Experimental Diphtheritic Intoxication I Blood Sugar, Lactic Acid and Nonprotein and Amino-acid Nitrogen

TABLE II

Average respiratory quotients obtained in normal and diphtheritic rabbits after different periods of fasting

Hours of starvation	Diphtheritic		Normal	
	Average respiratory quotient	Number of observations	Average respiratory quotient	Number of observations
24	81	4	84	3
48	80	4	82	4
72	78	3	76	4
96	80	2	76	5
120+	78	2	73	1

to make the values express surface area in the ordinary units. The omission of this procedure still allows comparison of the various animals in terms analogous to those which would be obtained with a more exact formula for surface area.

The respiratory quotients determined during the course of the intoxication do not suggest any gross interference with the burning of carbohydrates. It can be seen that after the development of a fasting status the respiratory quotients tend to remain with only slight exceptions at a fairly constant level, which averages between 78 and 80, a level well above that to be expected had there been any severe degree of insulin dysfunction.

In Table II the respiratory quotients obtained on the diphtheritic animals after similar periods of fasting have been averaged and compared with those obtained on the normal controls. The lack of any striking difference between the two sets of values is apparent.

It might be mentioned in passing, that no great change was noted in the total metabolic rate as the intoxication progressed. In other words diphtheria toxin when injected into rabbits does not exhibit any calorogenic activity.

DISCUSSION

Essentially the same approach for the investigation of the disturbance in carbohydrate metabolism during diphtheria has been utilized previously by Josephs (3), Hector (4) and Arloing and Laulanie (5).

Josephs determined the respiratory quotients in dogs by collection of the expired air over periods of from three to five minutes, followed by analysis of a sample in a Haldane gas apparatus. The results of four experiments were described. From the data presented it is evident that there was no difficulty in the oxidation of carbohydrates; the nonprotein respiratory quotients remaining about 0.80 throughout the course of the investigation. The dogs were subjected to only a mild degree of intoxication as evidenced by the relatively low blood nonprotein nitrogen levels and the prolonged periods of survival.

Arloing and Laulanie in 1895 studied the respiratory exchange by means of closed calorimetry in rabbits following the injection of large doses of toxin subcutaneously (death occurred in from 6 to 36 hours) Because of the overwhelming nature of the intoxication it is difficult to interpret the results Furthermore there was no suitable dietary control previous to the experiment Respiratory quotients ranging from 0.90 to 0.70 were obtained A tendency for a slight rise to occur during the first six to eight hours following the injection of toxin was noted

Hector in 1926 studied the respiratory quotients in six cases of diphtheria of varying degrees of severity, using a Fridericia apparatus He determined the respiratory quotient at frequent intervals following the administration of glucose by mouth From his results he concluded that there was no evidence of any inability to oxidize carbohydrates during the course of diphtheria

J. A. Johnston (6) has recently acquainted us with data similar to Hector's, on a diphtheria patient with severe myocardial involvement The observations, made on the 22d day of the disease, showed that the respiratory quotients following the intravenous administration of 20 grams of glucose rose from the pre-injection level of 0.84 to 0.90 at one hour and fell to 0.85 and 0.77 at the second and third hours respectively Comparing this with similar studies on normal children, Johnston interprets these results to signify an adequate response to the injected glucose These observations were made too late in the disease to be strictly comparable to our experimental results Similar studies, both early and late in the disease, would be valuable, especially if accompanied by observations of the blood nonprotein nitrogen, which seems to be the best objective measure of the severity of the intoxication

In Paper IV of this series (8), the results of the investigation of the serum electrolytes during diphtheritic intoxication in rabbits will be presented These studies demonstrate the frequent occurrence of a decrease in serum bicarbonate, chloride and sodium It is apparent that the development of acidosis while in the respiratory chamber would increase the respiratory quotient However, it is not likely that any marked change occurred during the relatively short period of observation Furthermore, it is unlikely that low respiratory quotients were so masked by developing acidosis as to bring about the substantial agreement in the respiratory quotients found in our experiments

The criticism which may be made of the work reviewed above, as well as of our experiments, is that by this type of investigation one can adequately demonstrate only severe grades of interference with carbohydrate oxidation The failure to find any evidence of this disability would, therefore, not preclude minor degrees of insulin insufficiency or dysfunction We believe this to be a just criticism and have duly considered it in framing our conclusions

SUMMARY AND CONCLUSION

1 Study of the respiratory quotients of rabbits during the course of diphtheritic intoxication produced by the intravenous injection of diphtheria toxin reveals no evidence of any gross disturbance in the oxidation of carbohydrates

2 It was not possible to demonstrate any significant changes in the total metabolic rate during the course of the intoxication

BIBLIOGRAPHY

- 1 Yannet, H, and Darrow, D C, *J Clin Invest*, 1933, xii, 767 Physiological Disturbances during Experimental Diphtheritic Intoxication I Blood Sugar, Lactic Acid and Nonprotein and Amino-acid Nitrogen
- 2 Marine, D M, *J Metab Research*, 1922, ii, 29 A Modified Haldane Open Circuit Apparatus for Measuring the Respiratory Exchange in New Born Babies and also in Rabbits and Cats
- 3 Josephs, H W, *Bull Johns Hopkins Hosp* 1927, xli, 400 The Metabolism Following Injection of Diphtheria Toxin in Dogs
- 4 Hector, F J *Lancet*, 1926, ii, 642 Carbohydrate Metabolism in Diphtheria
- 5 Arloing, S, and Laulanie F *Arch de physiol norm et path*, 1895, vii, 675 Introduction a la etude des troubles de la temperature des combustions respiratoires et de la thermogenese sous l'influence des toxines bacteriennes
- 6 Johnston, J A, Personal communication
- 7 Yannet H, and Darrow, D C, *J Clin Invest.*, 1933, xii, 779 Physiological Disturbances during Experimental Diphtheritic Intoxication II Hepatic Glycogenesis and Glycogen Concentration of Cardiac and Skeletal Muscle
- 8 Darrow, D C Yannet, H and Cary, M Katharine (To be published) Physiological Disturbances during Experimental Diphtheritic Intoxication IV Blood Electrolytes and Hemoglobin Concentrations

STUDIES OF TOTAL PULMONARY CAPACITY AND ITS SUBDIVISIONS I NORMAL ABSOLUTE AND RELATIVE VALUES

By ALBERTO HURTADO¹ AND CHARLES BOLLER

(From the Department of Medicine of the School of Medicine and Dentistry of the University of Rochester and the Medical Clinic of the Strong Memorial Hospital Rochester New York)

(Received for publication April 7, 1933)

In the investigation of the symptom, dyspnea by physiological methods studies of the pulmonary mechanisms have lagged behind those of other mechanisms involved. In most clinics the measurement of the vital capacity is frequently made, but rarely in conjunction with measurements of total pulmonary air and its subdivisions. No complete understanding of the problem can be attained without a complete study of the functions of the lungs in addition to those of the blood and the circulatory organs. The studies to be reported here were commenced as a preliminary to an investigation of the functional disability caused by various types of fibrosis of the lungs, particularly the pneumoconioses. At the outset it was discovered that data regarding lung capacity were available in only a small number of normal individuals, so small that no standards of normality could be established for comparison with measurements to be made in pathological conditions.

We will not attempt to make a historical review of the methods used in the determination of the lung volume and its subdivisions. A complete presentation of the subject, from this point of view, has been published very recently by Christie (4). We may mention that probably three factors have largely been responsible for hesitancy in accepting this procedure as a useful tool in clinical medicine: unreliability of the methods used, difficulty of using complicated methods in dealing with very sick patients, and finally lack of normal standards for comparative purposes. The first two drawbacks have been solved by the methods described by Van Slyke and Binger (12) and lately by Christie (4). The present study was undertaken in an attempt to furnish some information regarding the normal values of the pulmonary air and its subdivisions, to appreciate the character of its variations to establish a method which will permit the prediction of normal values in a given case and finally to apply the information gained from the normal observations to the study of cases of

¹ Travelling Fellow of the Rockefeller Foundation

chronic pulmonary pathology In this and subsequent papers we will present our results, together with a consideration of the related literature

NOMENCLATURE

A serious drawback to the proper comparison of different series of observations has been the use of different terms, and even different meanings for the same term It is therefore essential to adopt a single nomenclature in order to have a clear understanding of the subject We have adopted for our studies the classification proposed by Christie (4) with the exception of the replacement of the term "functional residual air" by mid capacity The classification may be briefly summarized as follows

Residual air is the amount of air remaining in the lungs after fullest possible expiration

Mid capacity is the amount of air remaining in the lungs after a normal expiration The term "functional residual air" introduced by Lundsgaard and Schierbeck (6), and used by Binger and Brow (3), is synonymous with this term It appears to be more convenient, however, to use the term mid capacity as being more descriptive and more commonly used The term "functional residual air" may be easily confused with residual air, or it may suggest that it is a subdivision of the latter Mid capacity represents the sum of the residual and reserve airs

Vital capacity is the amount of air expired in the fullest possible expiration following the deepest possible inspiration Vital capacity is the sum of the complementary and reserve volumes

Total capacity of the lungs is the sum of the residual air and the vital capacity

Complementary air is the volume of air inspired from the position of mid capacity to that of the maximum possible inflation It includes the tidal air

Reserve air is the amount of air expired from the mid capacity position to the maximum possible deflation

The character of this classification may be appreciated more readily in the diagram of Figure 1 Other classifications differ mainly in the definition of the mid capacity Panum (9), in 1868, regarded this as the volume of air in the lungs at a point mid-way between normal expiration and inspiration Most investigators have adopted his definition It seems better, however, to define mid capacity as proposed by Siebeck (11), who took into consideration the commonly accepted finding that the most constant level in any graphic respiratory tracing is found at the end of a normal expiration (Christie calls this level the "resting respiratory level") The level at which the mid capacity is placed in the different classifications changes the values given to the complementary and reserve volumes It will be noted that in the nomenclature adopted for this study the complementary air is the volume of air from the level of normal expiration up to

the limits of complete inspiration (consequently it includes the *tidal air* which is the amount of air moving in and out during quiet breathing) The reserve air is the volume of air between the level of normal expiration and the limits of complete expiration

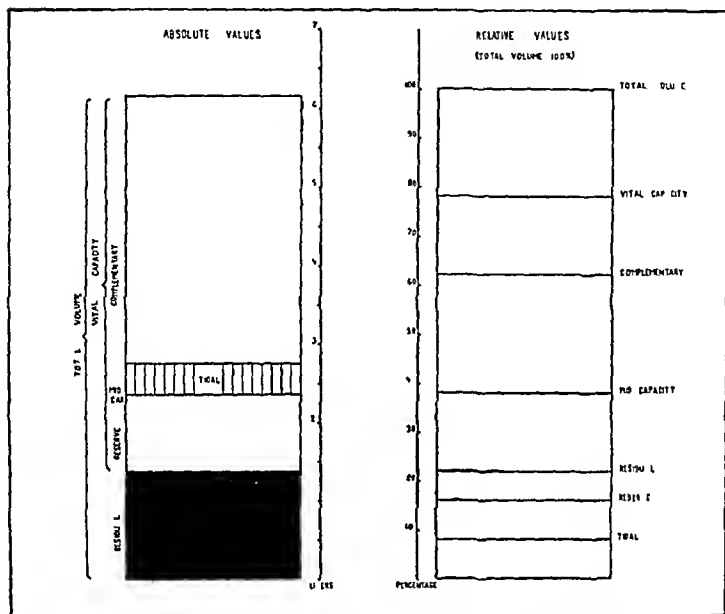


FIG 1 MEAN ABSOLUTE AND RELATIVE VALUES OF PULMONARY AIR IN 50 NORMAL MALES

METHODS

We have used Christie's method (4) of oxygen dilution without forced breathing. The details of this method have been fully described in this author's original description, so we will omit a detailed account. The spirometer employed was of the type described by Van Slyke and Binger (12) of nine liters capacity, provided with a solid core to decrease the dead space with a graduated millimeter scale and a recording pen (each millimeter on the scale being equivalent to a volume of 20 cc). A five way valve permitted easy and quick communication of the patient with the desired connection. The dead space of the spirometer, including rubber tubing soda lime container etc. was calculated according to the instructions given by Christie, and found to be 2600 cc. Care was taken to keep the same level of water in the spirometer by means of a small syphon mounted on a graduated scale. The oxygen used

was analyzed to discover inert gases acting as impurities. Five liters were admitted to the spirometer for each determination. The respiratory tracings were recorded on paper with millimeter rulings, mounted on a kymograph drum. From the tracings (the patient being under basal conditions) the basal metabolic rate may be calculated, if desired. All our results have been corrected to the volume corresponding to a temperature of 37°C , and complete saturation with water vapor. The main defect of this method, as pointed out by Christie, is the difficulty in making an accurate measurement of the oxygen consumption. Although in most cases a satisfactory base line is secured, there are cases in which breathing is somewhat irregular, and in consequence only an approximate determination of the oxygen consumption may be obtained. The error under these conditions may be considerable. In such cases we have used the following procedure for the determination of the *residual air*. A second spirometer containing about three or four liters of atmospheric air, and provided with a recording pen, is connected by means of a wide and short rubber tube to one of the outlets of the five way valve. The kymograph is placed so that a graphic tracing may be obtained from the excursions of this spirometer. The subject breathes through the five-way valve to the outside air for one or two minutes. Then he is rapidly connected with this spirometer. After three or four breaths, at the beginning of a normal expiration, he is suddenly asked to continue to expire as completely as possible to the level of the maximum deflation, and to hold his breath at this level for just the time (about a second) necessary to turn the valve connection to the regular mixing spirometer containing the measured amount of oxygen. Quiet breathing is continued for seven or eight minutes and respirations are recorded on the kymograph to which the recording pen of this spirometer is adjusted as soon as possible after the connection is made. At the end of this period the subject is again suddenly asked to continue as full an expiration as possible and to hold the breath at this level while the connection to the spirometer is quickly closed off. The oxygen consumption is calculated directly from the graduated scale of the spirometer (from the difference between the level after the known amount of oxygen was added and the level at the end of the determination). The respiratory tracing serves chiefly to detect any possible leakage or gross irregularity. It also gives a graphic record of the reserve air when the subject was asked to expire to the residual level at the beginning and end of the determination. The degree of the subject's cooperation can thus be readily determined by comparing these tracings, and proper corrections may be introduced, obviating, at least in part, the main error in the direct determination of the residual air. A third determination of the reserve air a few minutes later was sometimes used as a further check. A preliminary explanation usually suffices to obtain the desired cooperation. The rest of the calculation is made exactly as described by Christie, but of course the result obtained represents the residual air. This added to the reserve air will give the mid capacity.

Our gas analyses have been made in the Van Slyke manometric apparatus (10). This procedure is less time consuming than the use of the Haldane gas analysis apparatus. It has been found to give close agreement in duplicate analyses. All the determinations have been made with subjects in the recumbent position in bed, with two flat pillows for a head rest. In each case two determinations were made: one of mid capacity, following strictly Christie's method, and the other by the alternate method of determining the residual air according to the procedure just described. In most cases, when the base line for measurement of the oxygen consumption was satisfactory, a close agree-

ment was found between the volume of the residual air obtained directly and the one calculated by subtracting the reserve volume from that of the mid capacity. The vital capacity, reserve and complementary airs were determined immediately afterward in the same spirometer without change in the subject's position. After connection was again established with the spirometer, the subject was asked to breathe normally for one or two minutes and then while watching the graphic registration of his breathing he was asked to continue an expiration down to the maximum point of deflation to measure the reserve air. After quiet breathing was again resumed for a short time he was asked to continue an inspiration up to the maximum level of inflation thus measuring the complementary air. Finally after a short interval, we obtained a graphic tracing of the fullest possible inspiration followed by the maximum possible expiration (vital capacity). In normal people this vital capacity ought to be equal to the sum of the reserve and complementary volumes determined separately. Not infrequently small differences are found, possibly due to lack of cooperation.

MATERIAL

Determination of the total pulmonary air and its subdivisions has been made in 50 normal males, varying in age from 18 to 30 years and with a mean age of 23 years. The physical characteristics of these subjects are summarized in Table 1, from which it can readily be appreciated that no

TABLE 1
Physical characteristics of the subjects examined

	Mean	Standard deviation	Coefficient of variation	Variations
			per cent	
Age years	22.9 \pm 0.31*	3.3 \pm 0.22*	14.4	18-30
Body height cm	176.2 \pm 0.49	5.1 \pm 0.35	2.9	157.6-186.5
Body weight, kgm	72.5 \pm 1.07	11.2 \pm 0.76	15.5	52.8-104.0
Body surface area cm ²	187.8 \pm 1.19	12.5 \pm 0.84	6.6	152-219
Chest circumference cm	85.4 \pm 0.60	6.3 \pm 0.43	7.3	74-110
Chest volume liters	10.00 \pm 0.19	2.03 \pm 0.14	20.3	7.00-18.33
Chest index $\frac{\text{Depth}}{\text{Width}} \times 100$	68.5 \pm 0.55	5.8 \pm 0.39	8.4	58.3-84.4
Chest index $\frac{\text{Height}}{\text{Width}} \times 100$	70.8 \pm 0.71	7.3 \pm 0.49	10.3	50-84
Costal angle °	71.8 \pm 1.18	12.4 \pm 0.84	17.2	50-104

* Probable error

selective criteria were used in regard to the bodily appearance. The chest size, as judged from external measurements, also varies within wide limits as well as its shape (as indicated by the variations in the indices of Depth/Width and Height/Width and in the costal angle). This group of normal males although not very large in number, is accordingly representative and may be considered as a fair sample of this age period. The

subjects were students of the School of Medicine and the College for Men of the University of Rochester. In all cases a brief history of previous diseases and athletic activities was recorded, and the chest examined clinically and radiologically. The vast majority of the subjects were in non-fasting condition at the time of the determination, but all of them had a preliminary resting period of at least twenty minutes. The total number of observations was fifty-two. We have excluded from this report only two cases: in one the value for residual air was found to be a negative quantity for some unknown reason, and in the other case satisfactory co-operation could not be obtained.

TABLE 2
*Values of pulmonary capacity from the literature **

Absolute values

Pulmonary capacity	Mean	Standard deviation	Coefficient of variation	Variations
	<i>liters</i>	<i>liters</i>	<i>per cent</i>	<i>liters</i>
Total capacity	5.98	1.12	18.7	3.25-8.22
Vital capacity	4.46	0.87	19.5	2.31-5.95
Complementary air	2.60	0.63	24.2	1.61-3.95
Reserve air	1.98	0.51	25.7	0.70-2.92
Mid capacity †	3.70	0.66	17.8	2.23-5.08
Residual air	1.50	0.33	22.0	0.87-2.48

Relative values (Total capacity = 100 per cent)

	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Vital capacity	75.1	4.2	5.6	60-84
Complementary air	40.6	4.9	12.0	26-48
Reserve air	30.0	6.2	20.6	15-40
Mid capacity	60.7	4.0	6.6	52-69
Residual air	25.3	4.3	17.0	16-40

Relative values (Vital capacity = 100 per cent)

	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Complementary air	56.7	5.9	10.4	44-69
Reserve air	44.0	5.9	13.4	31-56
Mid capacity	81.2	8.7	10.7	63-97
Residual air	34.2	11.0	32.1	19-66

* Summary of 46 determinations made on male subjects collected from Lundsgaard and Van Slyke (7), Lundsgaard and Schierbeck (6), Binger (2) and Anthony (1).

† Volume of air in the lungs mid-way between a normal expiration and inspiration.

Normal values given in the literature

Records of normal values for the total capacity and its subdivisions are few, and it is difficult to evaluate them since the observations were made

with different techniques and under varying conditions. Information about the age and physical characteristics of the people examined was usually meager. Frequently values obtained in males and females have been combined so that it is difficult to separate the results according to sex. We have summarized in Table 2 all the determinations made on normal males since Lundsgaard and Van Slyke (7) reported 11 observations in 1918. No attempt has been made to include earlier data, since the methods used are open to question. We have included 11 cases of Lundsgaard and Van Slyke, 19 cases of Lundsgaard and Schierbeck (6) the largest series in the literature, 7 cases reported by Binger (2) and finally 9 cases from Anthony's report (1), making a total of 46 cases. The determinations have been made either standing or sitting up, and the classification used by the authors quoted places the mid capacity halfway between normal expiration and inspiration. In 11 cases, of the 19 reported by Lundsgaard and Schierbeck, we have found that the reported value of mid capacity is equal to the sum of the residual air and half the vital capacity, evidence that different criteria have been used in the calculation of that subdivision. From the series of Anthony we have included only the values reported for residual air. Table 2 has been arranged to present the summary of these data in absolute values for the different components of the total capacity, as well as their proportional relationship to the total volume and vital capacity. Comparison of results obtained from the literature with our findings is made elsewhere in this paper.

RESULTS

In Table 3 are summarized the determinations of the total pulmonary air and its subdivisions, indicating the mean values, the deviations from the mean and the extreme variations. The observed values are given, and relative values are expressed as percentages of the total volume and vital capacity.

Absolute values observed. The mean value of the *total capacity* in this series is 6.13 ± 0.08 liters, a figure very close to that of 5.98 liters, which is the corresponding mean of all the observations collected from the literature. There are wide variations from this mean. The standard deviation is 0.82 liter, with a coefficient of variation of 13.3 per cent, indicating that in our series there is a total range of variability of about 27 per cent from the mean value. Similar, and even higher, variability becomes evident from the study of the reported observations in the literature.

When we come to the *vital capacity* we also find, as others have found, a wide variation in the absolute values. With a mean value of 4.78 ± 0.06 liters it varies between 3.40 and 5.85 liters, with a total variation of about 25 per cent from the mean. The fact that all our subjects were young males possibly explains the higher mean value we obtained as compared with the one calculated from the observations of other investigators.

TABLE 3

Determinations of pulmonary capacity in 50 healthy males

Absolute values

Pulmonary capacity	Mean	Standard deviation	Coefficient of variation	Variations
	<i>liters</i>	<i>liters</i>	<i>per cent</i>	<i>liters</i>
Total capacity	6 13 \pm 0 08*	0 82 \pm 0 06	13 3	4 42-7 86
Vital capacity	4 78 \pm 0 06	0 59 \pm 0 04	12 3	3 40-5 85
Complementary air	3 79 \pm 0 05	0 52 \pm 0 04	13 7	2 41-4 93
Reserve air	0 98 \pm 0 02	0 26 \pm 0 02	26 5	0 26-1 58
Mid capacity	2 34 \pm 0 05	0 49 \pm 0 03	20 9	1 09-3 38
Residual air	1 36 \pm 0 04	0 38 \pm 0 03	27 9	0 81-2 16

Relative values (Total capacity = 100 per cent)

	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Vital capacity	78 0 \pm 0 41	4 3 \pm 0 29	5 5	68 4-85 5
Complementary air	61 9 \pm 0 51	5 3 \pm 0 36	8 5	51 7-78 9
Reserve air	16 2 \pm 0 39	4 1 \pm 0 28	25 3	5 0-30 3
Mid capacity	37 9 \pm 0 75	7 9 \pm 0 53	20 6	21 0-50 3
Residual air	22 0 \pm 0 41	4 3 \pm 0 29	19 5	14 5-31 6

Relative values (Vital capacity = 100 per cent)

	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Complementary air	79 4 \pm 0 50	5 2 \pm 0 35	6 5	63 1-94 1
Reserve air	20 6 \pm 0 52	5 4 \pm 0 36	26 2	5 9-36 9
Mid capacity	49 1 \pm 0 85	8 9 \pm 0 60	18 1	25 0-70 4
Residual air	28 2 \pm 0 70	7 3 \pm 0 49	25 8	16 9-46 0

* Probable error

The *mid capacity* also shows wide fluctuations. It varies between 1 09 and 3 38 liters, with a mean value of $2 34 \pm 0 05$ liters. It shows a total variation of about 42 per cent from the mean. Our results cannot be compared with those reported by other investigators on account of the difference in classification used. Binger and Brow (3) in nine determinations of the mid capacity (called "functional residual air" in their report), which was measured in a manner analogous to ours, obtained, however, an average value of 2 43 liters in very close agreement with the mean value of this series.

The volume of *residual air* varies markedly. Its lowest and highest values in our series are 0 81 and 2 16 liters and the mean value is $1 36 \pm 0 04$ liter, a figure slightly lower than 1 50 liter which is the mean value of the cases previously reported.

The *complementary* and *reserve volumes* show marked deviations from the corresponding mean values, especially the latter. The diagram of Figure 1 illustrates graphically the mean values obtained for the total capacity and its subdivisions.

Relative values It has been customary to express the values of the lung volume subdivisions as percentages of the total capacity. This is the more valuable method of expressing the results, as one may readily see from an inspection of the diagram of Figure 2. The vital capacity and

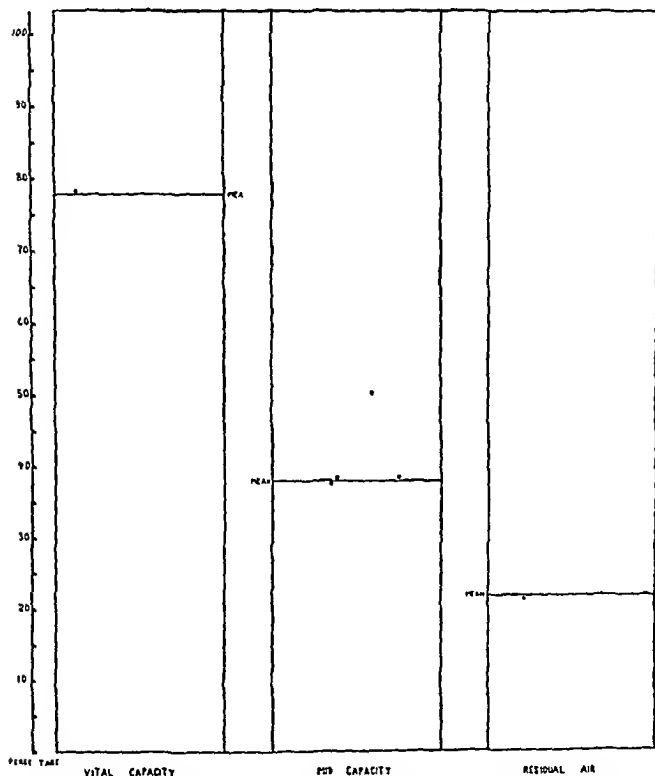


FIG 2 VITAL CAPACITY, TIDAL CAPACITY AND RESIDUAL AIR EXPRESSED IN PERCENTAGE OF THE TOTAL CAPACITY

The dots indicate 50 individual observations grouped about the mean values

capacity and residual air constitute more or less constant fractions of the total capacity in normal people, giving a sound basis for comparison with the possible variations of these ratios in pathological subjects. In the case of the vital capacity we find that it has a mean value representing 78

± 0.41 per cent of the total volume. The standard deviation is only 4.3 per cent, so all variations actually come within 12 per cent of that mean value. It appears to indicate a rather constant relationship of vital capacity to residual air in constituting the total lung capacity. It is significant to find that the same ratio (Vital capacity/Total capacity) $\times 100$ calculated from the cases reported in the literature has a mean value of 75.1 per cent, with a standard deviation of 4.2 per cent, that is a similar range of variability as found in our series. There is only one case (from the series of Lundsgaard and Van Slyke) in which this ratio was lower than 69 per cent.

The residual air, expressed in percentage of the total capacity, varied in our series from 14 to 31 per cent, with a mean value of 22.0 ± 0.41 per cent. In 96 per cent of the cases it formed from 15 to 30 per cent of the total volume. The data in Table 4 show that the residual air varies more

TABLE 4
Comparison of residual air, vital capacity and total capacity

$\frac{\text{Ratio Residual air}}{\text{Total capacity}} \times 100$	Residual air	Vital capacity	Total capacity
<i>per cent</i>	<i>liters</i>	<i>liters</i>	<i>liters</i>
14-16	0.90	4.80	5.71
17	1.13	4.79	5.91
20	1.28	4.74	6.02
23	1.53	4.92	6.45
26	1.68	4.68	6.36
29	1.96	4.26	6.22

Correlation coefficients

Ratio $\frac{\text{Residual air}}{\text{Total capacity}}$	and residual air	$+0.8740 \pm 0.0229^*$
	Idem and vital capacity	$+0.0164 \pm 0.0971$
	Idem and total capacity	$+0.3898 \pm 0.0823$

* Probable error

than the vital capacity. From the cases reported in the literature we obtained a mean value of 25.3 per cent in the ratio (Residual air/Total capacity) $\times 100$, and in the recent series of Anthony (1) the average of eight determinations (also made in the recumbent position) was 23.2 per cent.

The ratio (Mid capacity/Total capacity) $\times 100$ has a mean value of 37.9 ± 0.75 per cent and a standard deviation of 7.9 per cent. In 96 per cent of the cases a ratio was between 30 and 50 per cent. The complementary and reserve airs, expressed as percentage of the total volume, show moderate variations in the case of the former, and very wide fluctuations in the percentage of the latter. The close correlation of the total capacity with the vital capacity, mid capacity and residual air may also be appreciated from the high and significant correlation coefficients calculated in our series and presented in Table 5.

TABLE 5

Correlation between the different subdivisions

Capacities correlated	Correlation coefficient
Total capacity and vital capacity	+0.8950 ± 0.0188*
Total capacity and complementary air	+0.7889 ± 0.0361
Total capacity and reserve air	+0.4171 ± 0.0782
Total capacity and mid capacity	+0.8020 ± 0.0337
Total capacity and residual air	+0.7721 ± 0.0384
Vital capacity and complementary air	+0.8836 ± 0.0209
Vital capacity and reserve air	+0.4347 ± 0.0769
Vital capacity and mid capacity	+0.5748 ± 0.0634
Vital capacity and residual air	+0.4571 ± 0.0748
Complementary air and mid capacity	+0.3296 ± 0.0850
Complementary air and reserve air	+0.0195 ± 0.0951
Complementary air and residual air	+0.4731 ± 0.0735
Mid capacity and reserve air	+0.6191 ± 0.0587
Mid capacity and residual air	+0.8202 ± 0.0310
Residual air and reserve air	+0.1491 ± 0.0931

* Probable error

Few investigators have expressed the values of the subdivisions of the pulmonary air as a percentage of the vital capacity. There appears to be no special advantage in doing so, and perhaps it will be more convenient and simpler to use the total capacity as a basis for percentage estimations, rather than the vital capacity. It is interesting to notice the rather constant relationship between complementary air and vital capacity, the former constituting about 80 per cent of the latter.

Oxygen consumption and its relationship with ventilation per minute and vital capacity Efforts have been made by different investigators to correlate the oxygen consumption of the body with the ventilation in a given period of time in the hope of finding fixed ratios for normal people and possible deviations in pathological conditions. Knipping and Moncrieff (5) have investigated the so called "ventilation equivalent for oxygen" which may be calculated according to the formula (Minute volume respiration/ O_2 used per minute) $\times 100$. They report observations in 54 normal subjects (31 males and 23 females) and obtained an average value of 2.44 liters for this ratio, with variations ranging between 1.68 and 3.70 liters. In 19 cases of heart disease the results varied between 2.1 and 8.61 liters. Close examination of their findings does not indicate any value in such a ratio as a measure of respiratory efficiency. In 15 of our subjects in whom the determination of total capacity was done under basal conditions (rest and fasting) the calculation of that ratio gives an average of 2.66 with variations between 1.47 and 4.04. The coefficient of variation is very high and the correlation coefficient between oxygen consumption and ventilation per minute is not significant. Anthony (1) has found in normal subjects a close correlation between the vital capacity and the calories utilized per 24 hours. In the 15 cases already mentioned we obtained a very high coefficient of variation in the ratio (Vital capacity/ O_2 consump

- 3 Binger, C A L, and Brow, G R, J Exper Med, 1924, *xxxix*, 677 Studies on the Respiratory Mechanism in Lobar Pneumonia A Study of Lung Volume in Relation to the Clinical Course of the Disease
- 4 Christie, R V, J Clin Invest, 1932, *xi*, 1099 The Lung Volume and its Subdivisions I Methods of Measurement
- 5 Knipping, H W, and Moncrieff, A, Quart J Med, 1932, *i*, 17 The Ventilation Equivalent for Oxygen
- 6 Lundsgaard, C, and Schierbeck, K, Acta med Scandinav, 1923, *lxi*, 541 Untersuchungen über die volumina der Lungen IV Die Verhältnisse bei Patienten mit Lungenemphysem
- 7 Lundsgaard, C, and Van Slyke, D, J Exper Med, 1918, *xxvii*, 65 Studies of Lung Volume I Relation between Thorax Size and Lung Volume in Normal Adults
- 8 Meakins, J C, and Christie, R V, Ann Int Med, 1929-30, *iii*, 423 Lung Volume and its Variations
- 9 Panum, P L, Arch f d ges Physiol, 1868, *i*, 125 Untersuchungen über die physiologischen Wirkungen der comprimierten Luft
- 10 Peters, J P, and Van Slyke, D D, Quantitative Clinical Chemistry II Methods Williams & Wilkins, Baltimore, 1932
- 11 Siebeck, R, Deutsches Arch f klin Med, 1910, *c*, 204 Über die Beeinflussung der Atemmechanik durch krankhafte Zustände des Respirations- und Kreislaufapparates
- 12 Van Slyke, D D, and Binger, C A L, J Exper Med, 1923, *xxxvii*, 457 The Determination of Lung Volume without Forced Breathing

STUDIES OF TOTAL PULMONARY CAPACITY AND ITS SUBDIVISIONS II CORRELATION WITH PHYSICAL AND RADIOLOGICAL MEASUREMENTS

BY ALBERTO HURTADO¹ AND WALTER W FRAY

(From the Department of Medicine of the School of Medicine and Dentistry of the University of Rochester and the Medical Clinic and Division of Radiology of the Strong Memorial Hospital Rochester N Y)

(Received for publication April 7 1933)

In a previous paper (5) we have presented the results of 50 determinations of pulmonary capacity made in an equal number of healthy males. It was demonstrated that the relative capacities (expressed in percentages of the total volume) fluctuated within narrow limits, but that there were wide variations in the absolute figures found for the total volume and its subdivisions. This fact makes rather difficult the recognition of moderate and perhaps important, deviations in a given case and also prevents a clear understanding of any alterations in the relative values. Thus a low ratio $(\text{Vital capacity}/\text{Total volume}) \times 100$ may be caused by either increased residual air, low vital capacity, or a combination of both factors and the proper interpretation will be obtained only if normal values are available for comparison. To standardize the procedure it appears to be necessary to find a correlation between the pulmonary capacity and certain bodily characteristics, so that from a knowledge of the latter it may be possible to predict the normal volumes. If we have at our disposal reliable criteria to permit judgment of changes in absolute as well as in relative pulmonary capacity a clearer view of the underlying pathological physiology may be obtained. A basis may also be provided for the proper classification and grouping of cases of respiratory inefficiency in which defective alveolar ventilation is an important factor.

Although numerous investigations have been made to correlate the vital capacity to body or chest measurements there have been very few attempts to correlate these characteristics with the total pulmonary capacity or with any one of its subdivisions, other than vital capacity. Lundsgaard and Van Slyke (7) in 1918 found in a few cases a definite correlation between the total capacity, vital capacity, mid capacity and residual air, and the so called "chest volume," calculated from the external measurement of the three diameters of the chest obtained at the end of full inspiration and expiration under resting conditions. This investigation was later confirmed by Lundsgaard and Schierbeck (6) who found a similar relationship and also con-

¹ Travelling Fellow of the Rockefeller Foundation

cluded that the degree of chest expansion (as determined by external measurements) affects the prediction of the corresponding pulmonary capacity. But Binger and Brow (2) could not demonstrate such a correlation of pulmonary capacities, either with the external chest measurements, with the body height, or with the surface area, and suggested that the level of the diaphragm was the determining factor, since a long-chested type of individual with relaxed abdominal walls shows a greater total volume than the broad, deep-chested muscular type. These investigators stated that the measurement of the area of the lung fields on radiographic films gives a fairly satisfactory means for predicting pulmonary capacity in normal individuals, although they did not prove so in the pneumonic patients whom they were then investigating. No data were presented in their communication to support this conclusion. Binger (1), while studying cases of cardiac decompensation, predicted the normal pulmonary capacity by assuming a vital capacity of 2.5 liters per square meter of body surface, and from this the other volumes were calculated on the basis of the rather fixed relative values of the main subdivisions of the total volume.

We have made in our 50 cases a statistical study of the possible correlations of the pulmonary capacities with dimensions of the body and chest, and with radiological measurements. It was also our aim in this study to gain helpful information concerning the comparative value of the methods for the determination of the degree of chest expansion, a factor seldom properly evaluated clinically but undoubtedly of importance in a consideration of respiratory mechanics. To find the influence of the shape of the chest on the different subdivisions of pulmonary capacity was another purpose of the investigation.

METHODS

The body height and weight were recorded in all cases, and the surface area calculated from the DuBois charts. The following chest measurements were obtained in all subjects (without clothing) in the respiratory positions of full expiration, mid capacity (at the end of a normal expiration), and on full inspiration: (a) *Chest circumference*—measured at the level of the nipple line, just below the pectoral prominences, (b) *Lateral diameter* (width), the distance between the midaxillary lines at the level of the nipple, (c) *Anteroposterior diameter* (depth), the distance from the midsternal line, also at the level of the nipples, to the vertebral column, and (d) *Longitudinal diameter* (height), a distance from the upper end of the sternum, at the mid-line, to the junction of the body with the xiphoid process of this same bone. A pair of calipers (pelvimeter) was used in making the measurements.

The external "chest volume" in the three respiratory positions was calculated (according to the Lundsgaard and Van Slyke technique) from the product of the diameters at the corresponding positions.

Radiographs of the chest were obtained at the end of maximum expiration maximum inspiration, normal expiration and, in most instances, at the end of normal inspiration. These four exposures were obtained on two films in the following manner: one of the films was first exposed at the end of maximum expiration, the exposure time being about three-quarters of that in routine radiography of the chest. A brass filter, 0.2 mm in thickness, was then placed over the chest at the level of the diaphragm, and a second exposure of the same length and character was made on the same film at the end of maximum inspiration. This film when developed shows the bony thorax and the diaphragm at both phases of respiration. The object in using the brass filter is to prevent over-exposure of the chest particularly of the diaphragm, during the second portion of the exposure. Much the same effect can be obtained without the use of the brass filter by making the length of exposure at expiration approximately five times that at inspiration. We found it more convenient to use the filter and at the same time obtained better definition of the upper portion of the thorax on inspiration. The principles of this technique have been explained by Thomas (1931) (8).

It is of the greatest importance that both exposures be made at the proper phase of respiration, i.e. after maximal effort, since the data obtained are used for the prediction of normal pulmonary capacity. We have found it convenient to use a signal (movement of fingers of one of the patient's hands) for timing the exposures.

The second film was obtained in a similar manner, varying only the phase of respiration for the exposures to normal expiration and normal inspiration. It is extremely important that the exposure for expiration be made first, reversal of the order results in obliterating the shadow of the diaphragm at expiration.

The films were obtained in the recumbent position. The individual was placed in the prone position on a table, the top of which had been modified to accommodate a tunnel in which the film cassette could be slipped without altering the position of the subject. The target film distance was six feet. The exposure time was approximately $1/20$ second. The kilovoltage was varied according to the thickness of the chest. The milliamperage remained constant at 200 milliamperes.

Films obtained in this manner show the degree of expansion of the thorax and of the excursion of the diaphragm during maximal and quiet respiration. Because of the fact that such films do not impart any idea of the third dimension lateral films were tried during the early portion of the work in an attempt to define the anteroposterior diameter. The use of these were abandoned because of the difficulty in obtaining two photographic impressions of the upper portion of the chest on the same film.

In determining the size of the chest two linear dimensions of the chest were used: the transverse diameter and the height of the lung field (above

the level of the domes of the diaphragm) The transverse diameter was obtained at the level of the 9th interspace posteriorly, the measurement being taken from the inner aspects of the ribs The height of the lung fields was determined by measuring the vertical distance from the dome of the diaphragm to the plane of the first rib (EF expiration and GH inspiration, Figure 1)

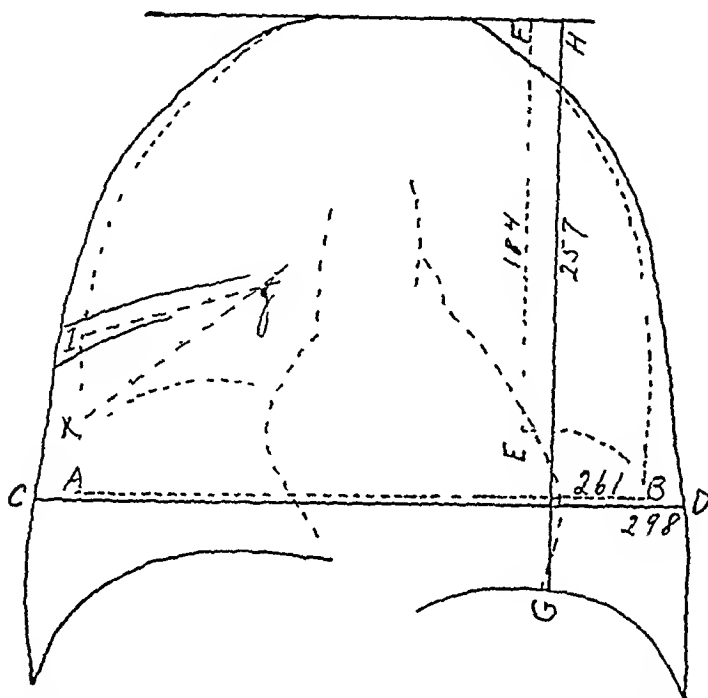


FIG 1 OUTLINES OF RADIOLOGICAL FILM WITH DOUBLE EXPOSURE TECHNIQUE (AT MAXIMUM EXPIRATION AND INSPIRATION)

Different measurements taken

These linear dimensions impart a very imperfect, or at least an incomplete, idea of the variation in chest size For this reason a planimeter, which recorded areas accurately up to 900 sq cm, was constructed The integrating wheel of this planimeter was approximately 7.5 cm in diameter, the tracing arm 50 cm in length, and the fixed arm 62 cm This instrument was calibrated by cutting circular grooves in a brass plate with diameters ranging from 10 to 40 cm The areas enclosed by these grooves were computed The planimeter was calibrated by comparing the readings on the scale after the point of the tracer arm completed the circle in the individual grooves with the respective areas as computed A graph was plotted from these determinations

With this instrument the areas of the lung fields were determined from the films obtained at the end of maximum and of normal expiration and inspiration The tracer arm was moved along the following course the

left axilla, left half of the diaphragm, right half of the diaphragm, right axilla, right apex and finally swept across the spine to the starting point at the left apex. The cardiac area, including the great vessels, was also obtained separately. The tracings obtained from films of a normal subject are illustrated in Figure 1. The area of the lung fields at maximum expiration was found to be 342 sq cm, and at maximum inspiration 571 sq cm. The difference represents the increase in area during maximal respiratory effort. Similarly, mensuration of the film taken at the end of normal expiration and inspiration yields data concerning the difference in area to be used in measuring reserve, complementary and tidal air, etc.

The extent of motion of the ribs was measured by determining the angular displacement of ribs during maximum respiratory effort. The 7th rib (posterior portion) was usually selected because it could be seen best in a film taken at maximum expiration and inspiration. Lines were drawn representing the direction of the same rib at expiration and inspiration and the angle between them measured. In the normal subject illustrated, this angle (IJK) becomes 22° .

Because of the curved course of the rib, its direction was determined by the slope of the outer two-thirds of its posterior portion.

It was thought that a better approximate value of the size of the chest cavity could be obtained from combined external and radiological measurements. We have multiplied in all cases the area of the lung fields (the area of the heart included) expressed in square centimeters, by the corresponding anteroposterior diameter (depth) of the chest measured externally in centimeters. The result is designated in this paper as the "radiological chest volume."

CORRELATION OF THE PULMONARY CAPACITY WITH PHYSICAL AND RADIOLOGICAL MEASUREMENTS

The correlation of the total capacity and its main subdivisions with body and chest dimensions and with radiological measurements has been summarized in Table I. It is convenient to mention that correlation coefficients are significant only when they exceed the probable error multiplied by three and that correlation is proportionally better as coefficients approach the value of 1.

In regard to the total capacity, we find that the highest correlation is obtained when the 'radiological chest volume' (at maximum inspiration) is used. It is correlated significantly although in a lesser degree with the area of lung fields (also at maximum inspiration) and with the body height. There is no useful correlation between the total pulmonary capacity and external chest measurements, the correlation coefficients are either valueless or very low.

The vital capacity is also better correlated with the "radiological chest volume." Its correlation of $+0.7174 \pm 0.0467$ is the highest of the

the level of the domes of the diaphragm) The transverse diameter was obtained at the level of the 9th interspace posteriorly, the measurement being taken from the inner aspects of the ribs The height of the lung fields was determined by measuring the vertical distance from the dome of the diaphragm to the plane of the first rib (EF expiration and GH inspiration, Figure 1)

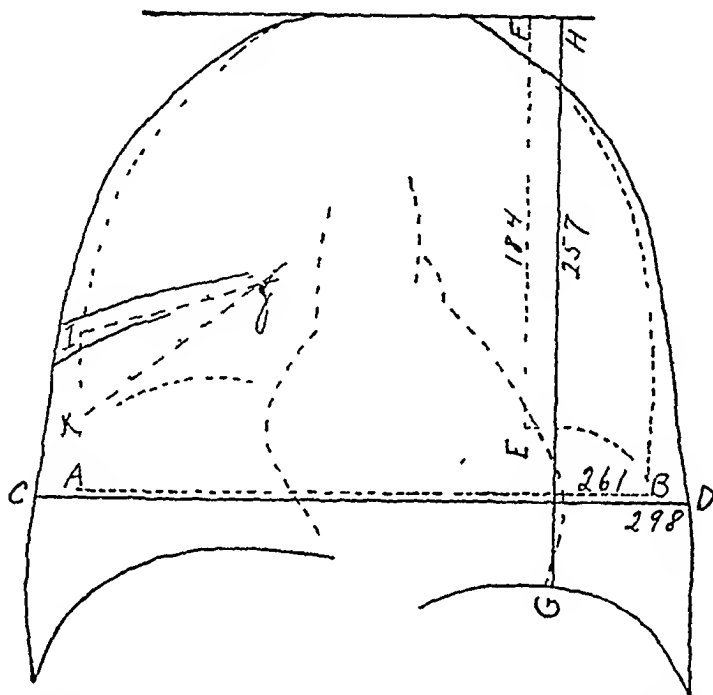


FIG 1 OUTLINES OF RADIOLOGICAL FILM WITH DOUBLE EXPOSURE TECHNIQUE (AT MAXIMUM EXPIRATION AND INSPIRATION)
Different measurements taken

These linear dimensions impart a very imperfect, or at least an incomplete, idea of the variation in chest size For this reason a planimeter, which recorded areas accurately up to 900 sq cm, was constructed The integrating wheel of this planimeter was approximately 7.5 cm in diameter, the tracing arm 50 cm in length, and the fixed arm 62 cm This instrument was calibrated by cutting circular grooves in a brass plate with diameters ranging from 10 to 40 cm The areas enclosed by these grooves were computed The planimeter was calibrated by comparing the readings on the scale after the point of the tracer arm completed the circle in the individual grooves with the respective areas as computed A graph was plotted from these determinations

With this instrument the areas of the lung fields were determined from the films obtained at the end of maximum and of normal expiration and inspiration The tracer arm was moved along the following course the

left axilla, left half of the diaphragm, right half of the diaphragm, right axilla, right apex, and finally swept across the spine to the starting point at the left apex. The cardiac area, including the great vessels, was also obtained separately. The tracings obtained from films of a normal subject are illustrated in Figure 1. The area of the lung fields at maximum expiration was found to be 342 sq cm, and at maximum inspiration 571 sq cm. The difference represents the increase in area during maximal respiratory effort. Similarly, mensuration of the film taken at the end of normal expiration and inspiration yields data concerning the difference in area to be used in measuring reserve, complementary and tidal air, etc.

The extent of motion of the ribs was measured by determining the angular displacement of ribs during maximum respiratory effort. The 7th rib (posterior portion) was usually selected because it could be seen best in a film taken at maximum expiration and inspiration. Lines were drawn representing the direction of the same rib at expiration and inspiration and the angle between them measured. In the normal subject illustrated, this angle (IJK) becomes 22° .

Because of the curved course of the rib, its direction was determined by the slope of the outer two thirds of its posterior portion.

It was thought that a better approximate value of the size of the chest cavity could be obtained from combined external and radiological measurements. We have multiplied in all cases the area of the lung fields (the area of the heart included) expressed in square centimeters, by the corresponding anteroposterior diameter (depth) of the chest measured externally in centimeters. The result is designated in this paper as the "radiological chest volume."

CORRELATION OF THE PULMONARY CAPACITY WITH PHYSICAL AND RADIOLOGICAL MEASUREMENTS

The correlation of the total capacity and its main subdivisions with body and chest dimensions and with radiological measurements has been summarized in Table I. It is convenient to mention that correlation coefficients are significant only when they exceed the probable error multiplied by three and that correlation is proportionally better as coefficients approach the value of 1.

In regard to the total capacity, we find that the highest correlation is obtained when the "radiological chest volume" (at maximum inspiration) is used. It is correlated significantly, although in a lesser degree, with the area of lung fields (also at maximum inspiration) and with the body height. There is no useful correlation between the total pulmonary capacity and external chest measurements, the correlation coefficients are either valueless or very low.

The vital capacity is also better correlated with the "radiological chest volume." Its correlation of $+0.7174 \pm 0.0467$ is the highest of the

TABLE I

Correlation of pulmonary capacities with physical and radiological measurements

Characteristics correlated	Correlation coefficient
Total pulmonary capacity and body weight	$-0.1267 \pm 0.0944^*$
Total pulmonary capacity and body height	$+0.5509 \pm 0.0661$
Total pulmonary capacity and body surface area	$+0.1644 \pm 0.0930$
Total pulmonary capacity and chest circumference (maximum inspiration)	$+0.1370 \pm 0.0944$
Total pulmonary capacity and chest volume (external) (maximum inspiration)	$+0.3271 \pm 0.0856$
Total pulmonary capacity and area of lung fields (maximum inspiration)	$+0.5848 \pm 0.0627$
Total pulmonary capacity and radiological chest volume (maximum inspiration)	$+0.6366 \pm 0.0566$
Vital capacity and body weight	$+0.1377 \pm 0.0930$
Vital capacity and body height	$+0.5452 \pm 0.0667$
Vital capacity and body surface area	$+0.2791 \pm 0.0876$
Vital capacity and chest circumference (maximum inspiration)	$+0.1591 \pm 0.0824$
Vital capacity and chest volume (external) (maximum inspiration)	$+0.3741 \pm 0.0816$
Vital capacity and area of lung fields (maximum inspiration)	$+0.6824 \pm 0.0509$
Vital capacity and radiological chest volume (maximum inspiration)	$+0.7174 \pm 0.0467$
Mid capacity and chest circumference (mid-position)	-0.2361 ± 0.0897
Mid capacity and chest volume (external) (mid-position)	-0.0554 ± 0.0957
Mid capacity and area of lung fields (mid-position)	$+0.5432 \pm 0.0674$
Mid capacity and radiological chest volume (mid-position)	$+0.4437 \pm 0.0769$
Residual air and chest circumference (maximum expiration)	-0.0737 ± 0.0944
Residual air and chest volume (external) (maximum expiration)	$+0.1065 \pm 0.0937$
Residual air and area of lung fields (maximum expiration)	$+0.3707 \pm 0.0823$
Residual air and radiological chest volume (maximum expiration)	$+0.3826 \pm 0.0809$

* Probable error

series, and consequently will be advantageously taken as the basis for the prediction of the normal pulmonary capacity in a given case. It is interesting to observe the almost valueless correlation between the vital capacity and the body surface area, a rather surprising finding as it has been widely accepted in clinics, following the work of West (9) in 1920. He found that this volume is better related to body surface area than to any other physical dimension, normal men having a vital capacity of about 2.5 liters per square meter. From the present observations and from previous experience, we think that there are wide variations in this relationship, it is usually found only when there is a normal balance between height and weight, and is not obtained in subjects who are either over or under weight. This has been so frequently observed in various clinics that it has limited the usefulness of the vital capacity determinations on the basis of body surface area, unless marked alterations are found.

The scatter of our fifty observations around the lines of correlation when the total capacity, the vital capacity and the different bodily thoracic and radiological measurements are compared, may be appreciated from a study of the Figures 2 and 3. It is plainly evident that the best correlation is

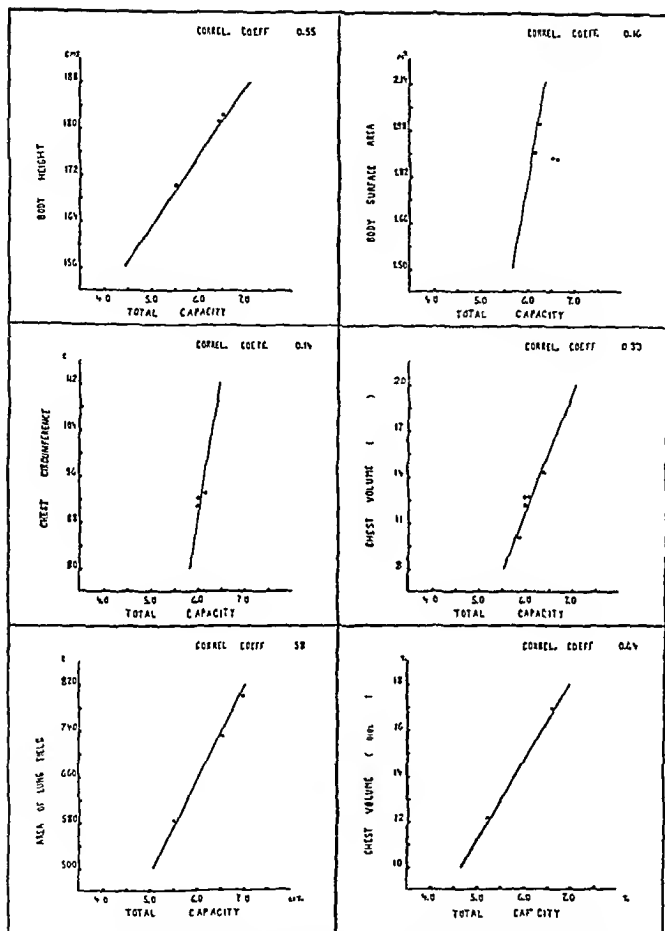


FIG 2 CORRELATION BETWEEN THE TOTAL PULMONARY CAPACITY AND PHYSICAL AND RADIOLOGICAL MEASUREMENTS

Lines represent ideal correlations (from the regression equations) Dots represent individual observations

TABLE I

Correlation of pulmonary capacities with physical and radiological measurements

Characteristics correlated	Correlation coefficient
Total pulmonary capacity and body weight	-0.1267 ± 0.0944*
Total pulmonary capacity and body height	+0.5509 ± 0.0661
Total pulmonary capacity and body surface area	+0.1644 ± 0.0930
Total pulmonary capacity and chest circumference (maximum inspiration)	+0.1370 ± 0.0944
Total pulmonary capacity and chest volume (external) (maximum inspiration)	+0.3271 ± 0.0856
Total pulmonary capacity and area of lung fields (maximum inspiration)	+0.5848 ± 0.0627
Total pulmonary capacity and radiological chest volume (maximum inspiration)	+0.6366 ± 0.0566
Vital capacity and body weight	+0.1377 ± 0.0930
Vital capacity and body height	+0.5452 ± 0.0667
Vital capacity and body surface area	+0.2791 ± 0.0876
Vital capacity and chest circumference (maximum inspiration)	+0.1591 ± 0.0824
Vital capacity and chest volume (external) (maximum inspiration)	+0.3741 ± 0.0816
Vital capacity and area of lung fields (maximum inspiration)	+0.6824 ± 0.0509
Vital capacity and radiological chest volume (maximum inspiration)	+0.7174 ± 0.0467
Mid capacity and chest circumference (mid-position)	-0.2361 ± 0.0897
Mid capacity and chest volume (external) (mid-position)	-0.0554 ± 0.0957
Mid capacity and area of lung fields (mid-position)	+0.5432 ± 0.0674
Mid capacity and radiological chest volume (mid-position)	+0.4437 ± 0.0769
Residual air and chest circumference (maximum expiration)	-0.0737 ± 0.0944
Residual air and chest volume (external) (maximum expiration)	+0.1065 ± 0.0937
Residual air and area of lung fields (maximum expiration)	+0.3707 ± 0.0823
Residual air and radiological chest volume (maximum expiration)	+0.3826 ± 0.0809

* Probable error

series, and consequently will be advantageously taken as the basis for the prediction of the normal pulmonary capacity in a given case. It is interesting to observe the almost valueless correlation between the vital capacity and the body surface area, a rather surprising finding as it has been widely accepted in clinics, following the work of West (9) in 1920. He found that this volume is better related to body surface area than to any other physical dimension, normal men having a vital capacity of about 2.5 liters per square meter. From the present observations and from previous experience, we think that there are wide variations in this relationship, it is usually found only when there is a normal balance between height and weight, and is not obtained in subjects who are either over or under weight. This has been so frequently observed in various clinics that it has limited the usefulness of the vital capacity determinations on the basis of body surface area, unless marked alterations are found.

The scatter of our fifty observations around the lines of correlation when the total capacity, the vital capacity and the different bodily thoracic and radiological measurements are compared, may be appreciated from a study of the Figures 2 and 3. It is plainly evident that the best correlation is

found in the case of the "radiological volume" and (a) the total capacity (Fig 2) and (b) the vital capacity (Fig 3)

When we come to the mid capacity we find a less perfect correlation with radiological data and a complete lack of relationship with external chest measurements. It is correlated best with the area of lung fields (radiological exposure at the end of a normal expiration), a fact worth taking into account in investigations where this subdivision of total volume is the only one that was determined and where knowing its normal value is desirable. The residual air is in very low correlation with radiological measurements, and in no correlation with external chest measurements.

From these observations it appears that we have been unable to confirm the early work of Lundsgaard and Van Slyke, and Lundsgaard and Schierbeck in demonstrating a correlation between the total capacity and its main subdivisions with the "chest volume" calculated from the three external diameters of the chest. This conclusion could have been predicted for it is evident that although the lateral and anteroposterior diameters may represent the dimensions of the chest in these planes the measurement of its height is not identical with the length of the sternum, and cannot indicate the level of the diaphragm, which varies widely from and independently of the sternal length. The "chest volume" calculated from these measurements yields consequently a doubtful value of the true volume of the chest cavity. Theoretically, it appeared to be a sounder assumption that multiplying the area of the lung fields measured in radiological films and the anteroposterior diameter of the chest, determined externally, would secure a value more accurately representative of the true size of the chest cavity. This assumption has been verified, for in this way a better correlation has been found between the volume of the lungs and that of the chest.

PREDICTION OF THE NORMAL CAPACITY OF THE LUNGS

It has now been shown that in the total series of correlations between the total pulmonary capacity and its components and the physical and radiological measurements the highest and most significant correlation coefficient has been observed between the vital capacity and the "radiological chest volume." This fact indicates that if the chest volume is known in a given case the vital capacity may be predicted with a fair degree of accuracy by means of the regression formula derived from the correlation coefficient.² Other fractions of the total capacity may be inferred from the percentage values which the vital capacity, the mid capac-

² The regression formula derived from the correlation coefficient between the mid capacity volume and the area of lung fields (also at the same respiratory position) is as follows

Mid capacity (liters) = (area of lung fields in cms \times 0.0042) + 0.33

³ The regression formula is

Vital capacity (liters) = (radiological chest volume (liters) \times 0.24) + 1.22

anteroposterior diameters vary widely, it is impossible therefore in a given case to justify the use of these measurements. It may be said in a general way that a circumferential expansion of less than 6 cm. is to be considered as reduced. No correlation could be found between pulmonary capacity and chest expansion determined by external measurements.

If on the other hand we measure from radiological films the areas of the lung fields at maximum expiration and inspiration the relation between the two varies within definite limits. The ratio (Area at maximum expiration/Area at maximum inspiration) $\times 100$ has, in our series, a mean value of 62.2 with a total deviation from the mean of less than 15 per cent. It appears that this ratio may, therefore, be used as a means of appreciating the degree of chest expansion. If it is higher than 72.0 we may suspect that a definite reduction in excursion has taken place. This ratio it must be remembered although it takes into consideration the diaphragmatic and lateral expansion of the chest neglects expansion in the anteroposterior diameter. But since as we have found, the mean values of the lateral and anteroposterior chest expansion are practically the same, the error will be more or less constant. As a matter of fact, we have found, in a group of cases of chronic pulmonary disease this similarity of expansion in the two planes.

The ratio (Area at maximum expiration/Area at maximum inspiration) $\times 100$ is higher in people with a long narrow chest and is chiefly altered by changes in the diaphragmatic excursion. It is interesting to find a relationship between this ratio and the relative values of the pulmonary capacity. In this series of normal cases the correlation coefficient between the ratios (Area at maximum expiration/Area at maximum inspiration) $\times 100$ and (Residual air/Total volume) $\times 100$ is significant $+0.4015 \pm 0.0796$ indicating that alterations in one ratio tend to be accompanied by similar and proportional changes in the other. We will show in another communication that this relationship is considerably more evident in pathological cases in which when expansion is diminished the residual air is high. This relationship between chest expansion and relative pulmonary capacity suggests that correction of the latter is necessary if expansion is diminished. A consequence of correcting such a value may be the neglect of a pathological factor so that a value significantly altered in fact may appear to be normal. It will be more helpful to have both ratios for the proper interpretation of a case rather than to modify one by means of the other.

Additional information concerning chest expansion may be obtained from a study of other measurements taken from the same radiological film. The diaphragmatic excursion, the lateral expansion of the chest and the degree of movement of the rib although showing considerable normal variations may be considered reduced when they move less than 4 cm., 2 cm. and 12° respectively.

SHAPE OF THE CHEST AND LUNG VOLUME

In all of our 50 cases two different indices have been calculated to determine the relationship between the shape of the chest and changes in pulmonary capacity and chest expansion. The index $(\text{Depth}/\text{Width}) \times 100$ will indicate whether the chest is of the rounded or flat type. There are wide variations in this index from 58 to 84 with a mean value of 68.5 (Table III). In comparing this index with the different absolute and rela-

TABLE III
Shape of the chest

Indices	Mean	Standard deviation	Coefficient of variation	Variations
			<i>per cent</i>	
$\frac{\text{Depth}}{\text{Width}} \times 100$	$68.5 \pm 0.55^*$	$5.8 \pm 0.39^*$	8.4	58.3-84.4
$\frac{\text{Height}^\dagger}{\text{Width}} \times 100$	70.8 ± 0.71	7.3 ± 0.49	10.3	50-84

* Probable error

† This measurement represents the average height of both sides of the diaphragm (at mid capacity) as determined on the radiological films

tive volumes, we have not found a significant correlation to indicate that variations in the pulmonary capacity may be attributed to this factor. There was no correlation, either, between this index and the degree of chest expansion, except that the movement of the ribs is less marked when the chest index is high that is to say in rounded chests.

The index $(\text{Height}/\text{Width}) \times 100$ (both diameters being measured on x-ray films taken at mid capacity) distinguishes two types of chest: broad, muscular ones with a high diaphragm (hypersthenic type), and slender and long narrow ones with a low diaphragm (asthenic type). In the former the index is low and in the latter high. We have found that the shape or type of the chest from this point of view has a definite influence upon the pulmonary capacity, and that this is reflected chiefly in changes in the volume of reserve air. In our series of normal males, it was not an uncommon observation to find a rather low vital capacity (as compared with the calculated value) in the hypersthenic type of individual due to the small volume of reserve air. The correlation coefficient between the index $(\text{Height}/\text{Width}) \times 100$ and the reserve air was $+0.4133 \pm 0.0803$ which is significant. In the three cases in which the lowest indices were obtained (50, 52 and 58) the observed reserve air was 0.26, 0.50 and 0.38 liters respectively.

The radiological chest volume was considerably larger in those individuals with long narrow chests and low diaphragms. They exhibited correspondingly the highest total pulmonary capacity. This fact stands in

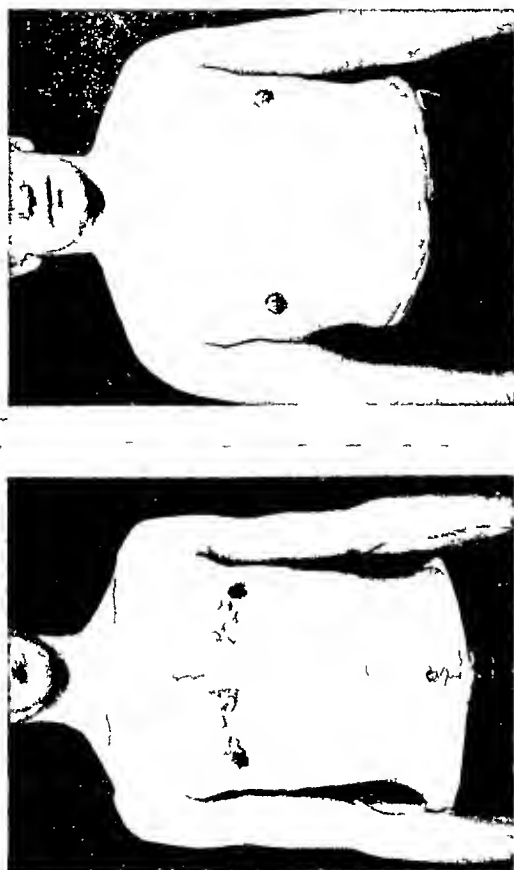


FIG. 5. ASTHENIC (LEFT) AND HYPERSTHENIC TYPES OF CHEST

The latter has a smaller total volume and a marked decrease in the volume of reserve air as compared with the asthenic chest. The diaphragmatic level is much higher in the hypersthenic chest.

sharp contrast with what one might judge clinically, because men with broad muscular and apparently big chest cavities are regarded as having greater pulmonary volumes compared with the other type. The reverse is, however, the case, indicating the importance of taking into consideration the diaphragmatic level in calculating the pulmonary capacity in a given case.

Comparing the chest expansion in hypersthenic individuals (broad muscular chests with high diaphragms) we did not find any significant relationship between this and the ratio $(\text{Area at maximum expiration} / \text{Area at maximum inspiration}) \times 100$ and the diaphragmatic excursion. There was, however, almost no lateral expansion of the chest during the determination of the reserve air (a forced expiration from the mid capacity level) in contrast with other cases in which an appreciable expansion was noticed and measured.

SUMMARY AND CONCLUSIONS

In a preceding paper (5) have been presented the values of pulmonary capacity found in observations made on 50 healthy males. In the present communication we have discussed the correlation between the total capacity and its main subdivisions with height, weight, surface area and external and radiological measurements of the thorax. It has been shown that they may be best calculated in a given case from the so-called "radiological chest volume". The method of calculation has been fully presented, comparison of the observed and the calculated volumes shows a very close correspondence. The application of these observations permits one to appreciate pathological deviations.

Studies have been made of the normal variability in the degree of expansion of the chest, which is best exhibited in measurements of the chest film taken by means of a standard technique which has been described. The same film, together with the measurement of the anteroposterior diameter of the chest at maximum inspiration, furnishes all necessary information for the calculation of a given pulmonary capacity if correlation coefficients and regression formulae are used.

The influence of the shape of the chest on the pulmonary capacity and on the degree of expansion of the chest has also been investigated.

The observations presented lead to the following conclusions:

1. When the total pulmonary capacity and its main subdivisions are calculated on the basis of the "radiological chest volume" at maximum inspiration, the following deviations in the observed values (as compared with the calculated ones) are considered to be significant: a difference of over 15 per cent in the total pulmonary capacity and vital capacity, and of 30 per cent and 40 per cent in the mid capacity and residual air respectively.

2. If the ratio $(\text{Area at maximum expiration} / \text{Area at maximum inspiration}) \times 100$ is higher than 72.0, a reduction in chest expansion is

indicated. Further evidence of deficient expansion is obtained if the diaphragmatic excursion, the lateral expansion and the degree of movement of the rib are found to be less than 4 cm, 2 cm and 12° respectively.

3. There is a certain correlation between the degree of chest expansion (as appreciated by the ratio mentioned) and the relative proportions of subdivisions of the pulmonary capacity. Deficient expansion tends to be accompanied by a higher percentage of the residual air in relation to the total capacity.

4. There is a relationship between the shape of the chest and the capacity of the lungs. Individuals with broad muscular chests and high diaphragms (hypersthenic type) usually present low volumes of reserve air as compared with long and narrow chested individuals with low diaphragms (asthenic type). The latter have larger thoracic capacity and consequently larger pulmonary capacity.

5. From the observations made on normal males it is possible to detect pathological changes in the absolute and relative pulmonary capacity in a given case. The importance of recognizing such alterations is obvious.

BIBLIOGRAPHY

1. Binger, C. A. L. J. Exper. Med. 1923 *xxxviii*, 445. The Lung Volume in Heart Disease.
2. Binger, C. A. L. and Brow, G. R. J. Exper. Med. 1924 *xxxix*, 677. Studies on the Respiratory Mechanism in Lobar Pneumonia. A Study of Lung Volume in Relation to the Clinical Course of the Disease.
3. Bolir, C. Deutsches Arch. f. klin. Med. 1907 *lxxxviii*, 385. Die funktionellen Änderungen in der Mittellage und Vitalkapazität der Lungen.
4. Hurtado, A. Am. J. Phys. Anthropol. 1932, *xvii*, 137. Respiratory Adaptation in the Indian Natives of the Peruvian Andes. Studies at High Altitude.
5. Hurtado, A. and Bolir, C. J. Clin. Invest. 1933 *xii*, Studies of Pulmonary Capacity and its Subdivisions. I. Normal Absolute and Relative Values.
6. Lundsgaard, C. and Schnerbeek, K. Proc. Soc. Exper. Biol. and Med. 1922-23 *xx*, 160. Studies on Lung Volume. VII. Relation of Size of Chest to Lung Volume.
7. Lundsgaard, C. and Van Slyke, D. D. J. Exper. Med. 1918 *xxvii*, 65. Studies of Lung Volume. I. Relation between Thoracic Size and Lung Volume in Normal Adults.
8. Thomas, H. Am. J. Roentgenol. 1931 *xxxi*, 878. A Graphic Method of Recording the Respiratory Excursion of the Diaphragm.
9. West, H. F. Arch. Int. Med. 1920 *xx*, 306. Clinical Studies on the Respiration. VI. A Comparison of Various Standards for the Normal Vital Capacity of the Lungs.

STUDIES OF TOTAL PULMONARY CAPACITY AND ITS SUBDIVISIONS III CHANGES WITH BODY POSTURE

By ALBERTO HURTADO¹ AND WALTER W. FRAY

(From the Department of Medicine of the School of Medicine and Dentistry of the University of Rochester and the Medical Clinic and Division of Radiology of the Stroud Memorial and Rochester Municipal Hospitals Rochester New York)

(Received for publication April 7 1933)

It has been known for many years that the posture of the body has an influence on pulmonary capacity. More than 25 years ago Bohr (3) and Plesch (10) found that the residual air increases in the recumbent position. Christie and Beams (6) in 1922 observed a reduction of 5.5 per cent in the vital capacity in the latter position and Wilson (11) a few years later demonstrated that this is brought about by a marked reduction in the reserve air. Binger and Brow (2) in 1924 found a considerable decrease in the functional residual air (mid capacity) with subjects flat in bed. The average reduction in nine cases was 0.80 liters. In recent years Anthony (1) reported practically no change in the volume of residual air depending on different positions of the body but confirmed the previous finding that there is marked reduction in the reserve air in the recumbent position and pointed out that the volume of complementary air changes in the opposite direction, it increases on assuming this position. Callhoun, Cullen and Harrison et al. (5) in 1931 found similar influences due to the position of the body on the pulmonary capacity.

We have shown in a previous paper (8) that there is a definite correlation between the total capacity and its subdivisions and the size of the chest cavity. It will be interesting therefore to discover whether changes in position are also accompanied by similar and proportional alterations in size and expansion of the chest. Hamilton and Morgan (7) in a recent communication reported that in five normal subjects there was no change in the volume of residual air in the recumbent position but they observed a moderate decrease in the vital capacity and consequently a corresponding diminution in the total capacity when lying was compared with the sitting posture. These investigators measuring the lateral and anteroposterior diameters of the chest found a moderate increase when the subjects were recumbent but failed to find consistent changes in the level of the diaphragm. From their observations they concluded that the chest cavity was larger when the body is recumbent and that the reduction in total volume

¹ Travelling Fellow of the Rockefeller Foundation

and vital capacity in this posture possibly is brought about by accumulation of blood in the pulmonary circuit

There is evidence in the literature to show that when recumbent there is diminution in the size of the cavity of the chest. Briscoe (4) from careful observations concluded that in this position there is a considerable reduction and that this is affected chiefly by an elevation of the diaphragm, while the lateral and anteroposterior diameters of the chest show very slight increase. Livingstone (9) in 1928 reported that in normal individuals the capacity of the chest is least in the supine posture and that this corresponds with a higher level of the diaphragm and with practically no change in the diameters of the thorax.

To study this matter further, we have made observations of 10 healthy males (average age 23 years) regarding the influence of posture on the capacity of the lungs and the size and expansion of the chest. The determinations of pulmonary capacity in the sitting and recumbent positions were made with a few minutes interval. Radiographs of the chest were obtained at maximum and at normal expiration and inspiration. The methods and technique employed in these observations and the different measurements obtained from the radiographs have already been described (8).

CHANGES IN PULMONARY CAPACITY WITH BODY POSTURE

The variations found (Table I) are interesting and, in general, agree with the work of previous investigators. When subjects assume the recumbent position there is, as compared with the sitting posture, a moderate reduction in the total capacity, vital capacity, and residual air (the average diminutions are 9.0, 6.6, and 14.5 per cent respectively) with more marked changes in the reserve air and mid capacity (28.2 and 41.7 per cent). The only volume which shows an alteration in the opposite direction is the complementary air. Its increase (11.1 per cent) is, however, proportionally lower than the decrease in the reserve air, so that the vital capacity is nevertheless reduced.

In the course of our investigation we also determined the vital capacity of 32 healthy males in the erect and lying positions. The results of these observations closely agree with the corresponding changes found in the ten cases. The average vital capacity in the erect posture was 5.06 liters while in the lying position it was 4.77 liters, a difference of 0.29 liters, a reduction of 5.7 per cent.

The alterations in the absolute values of some of the subdivisions on assuming the recumbent posture are of course reflected in their relative values because the total volume is proportionally less altered. There are very slight changes in the ratios (Vital capacity/Total volume) and (Residual air/Total volume) but on the other hand there are marked reductions in the ratios of reserve air and mid capacity to the total volume.

TABLE I

Changes in pulmonary capacity according to posture (mean of 10 observations)

Determinations	Means		Variation	
	Sitting	Lying	per cent	liters
	<i>liters</i>	<i>liters</i>		
Total pulmonary capacity	6 64±0 18	6 04±0 19*	-9 0	0 60
Vital capacity	4 99±0 12	4 66±0 14	-6 6	0 33
Complementary air	3 32±0 10	3 69±0 12	+11 1	0 37
Reserve air	1 68±0 04	0 98±0 06	-41 7	0 70
Mid capacity	3 33±0 14	2 39±0 11	-28 2	0 94
Residual air	1 65±0 10	1 41±0 09	-14 5	0 24
Ratios	per cent	per cent		
Vital capacity/Total volume	75 4±1 14	76 9±1 01	+1 9	
Complementary/Total volume	49 6±1 15	60 8±0 96	+22 5	
Reserve/Total volume	25 3±0 83	16 2±0 96	-35 9	
Mid capacity/Total volume	49 9±1 15	39 2±0 95	-21 4	
Residual/Total volume	24 5±1 06	23 0±1 13	-6 1	
Complementary/Vital capacity	64 8±1 95	78 5±1 19	+21 5	
Reserve/Vital capacity	33 5±1 06	21 0±1 12	-37 3	
Mid capacity/Vital capacity	66 7±2 47	51 3±1 78	-23 0	
Residual/Vital capacity	33 1±2 05	30 4±1 85	-8 1	

* Probable error

The complementary air increases and therefore its ratio is increased. The complementary air makes up a bigger part of the vital capacity and the reserve air the other component is correspondingly reduced.

The changes just mentioned in the absolute and relative values of the pulmonary capacity and its subdivisions according to posture indicate the necessity of making all determinations under similar and standard conditions to permit proper comparison. This fact has often been overlooked in the past.

INFLUENCE OF POSTURE ON SIZE AND EXPANSION OF THE CHEST

The different measurements obtained from the radiological films taken in the sitting and recumbent postures are summarized in Table II. Considering first the changes occurring in the size of the chest it will be noted that when recumbent the level of the diaphragm is distinctly higher, and that this is more marked at mid capacity (after a normal expiration). It is also higher at maximum expiration and inspiration but to a much smaller degree. The actual measurements of the height of the right and left sides of the diaphragm show that at mid capacity they are 5.6 and 4.1 cm. higher than in the sitting position. The higher level attained is slightly more marked in the right diaphragm.

TABLE II
Changes in size and expansion of the chest with body posture
(From radiological observations—mean of 10 cases)

Measurement	Means		Variations		Measurement	Means		Variations	
	Sitting, units	Lying, units	per cent	units		Sitting, units	Lying, units	per cent	units
Height of diaphragm At full expiration—right cm left	18.7 ± 0.56*	17.1 ± 0.17	-8.5	-1.6	Excursion of diaphragms Reserve—right cm left	5.0 ± 0.37	0.7 ± 0.12	-86.0	-1.3
	20.5 ± 0.53	19.2 ± 0.51	-6.3	-1.3		3.9 ± 0.32	0.83 ± 0.11	-78.1	-2.97
At mid capacity—right cm left	22.9 ± 0.65	17.3 ± 0.13	-24.1	-5.6	Complemental—right cm left	1.5 ± 0.25	6.1 ± 0.25	+326	+1.9
	23.6 ± 0.71	19.5 ± 0.26	-17.3	-4.1		2.1 ± 0.28	6.1 ± 0.28	+166	+1.0
At full inspiration—right cm left	24.8 ± 0.50	23.6 ± 0.46	-1.8	-1.2	Total—right cm left	6.0 ± 0.21	6.5 ± 0.21	+8.3	+0.5
	26.7 ± 0.50	25.8 ± 0.16	-3.3	-0.9		6.1 ± 0.19	6.6 ± 0.21	+8.2	+0.5
Width of chest At full expiration cm At mid capacity cm At full inspiration cm	26.9 ± 0.19	27.3 ± 0.28	+1.1	+0.4	Expansion in chest width Reserve cm Complemental cm Total cm	0.7 ± 0.09	1.2 ± 0.15	+71.1	+0.5
	27.6 ± 0.19	28.1 ± 0.31	+2.9	+0.8		3.4 ± 0.19	1.7 ± 0.19	-50.0	-1.7
	30.7 ± 0.29	30.3 ± 0.24	-1.3	-0.1		3.8 ± 0.18	3.0 ± 0.18	-21.0	-0.8
Area of lung fields At full expiration cm ² At mid capacity cm ² At full inspiration cm ² Cardiac area cm ²	128 ± 15.5	198 ± 18.0	-7.0	-30	Changes in arcs of lung field Reserve cm ² Complemental cm ² Total cm ² Rib rotation degrees	110 ± 9.21	15 ± 2.75	-67.8	-9.5
	85 ± 20.6	430 ± 14.0	-22.4	-125		104 ± 8.21	202 ± 10.1	+91.2	+9.8
	67.1 ± 17.1	6.39 ± 16.7	-1.7	-32		243 ± 9.55	241 ± 9.39	-0.8	-2
	157 ± 3.17	152 ± 2.96	-1.2	-2		20 ± 0.99	18 ± 1.24	-10	-2
					Area at maximum expiration		Area at maximum inspiration		
					X 100		Ratio		
					63.6 ± 1.24		62.1 ± 1.11		-1.5

* Probable error

It is interesting to see that the lateral dynamometer of the chest shows a very slight change, less than one centimeter, when the posture is varied from sitting to recumbency. We have not measured the anteroposterior diameter but from other investigations already cited it probably shows no change.

The size of the areas of lung fields shows similar alterations. At mid capacity it is reduced 22.4 per cent. At maximum expiration and inspiration the areas are slightly smaller although the decrease is only 7.0 and 4.7 per cent respectively.

From these observations it appears that the alterations in the size of the chest are of similar character to the variations in pulmonary capacity. We have seen that the volume of mid capacity is most markedly reduced (as compared with the total volume and residual air) and that similar alteration occurs in the radiological area of the lungs at this respiratory position. We have found that the decrease in pulmonary capacity is somewhat more marked however than the changes in the radiological area of the lungs. The total volume is reduced 9.0 per cent while the area at full inspiration is only 7.0 per cent smaller. The mid capacity decreases 28.2 per cent while the corresponding area shows a reduction of 22.4 per cent and finally the residual air decreases 14.5 per cent and the area at maximum expiration has diminished only 4.7 per cent in size. Does this mean that part of the alveolar space is encroached upon by the increased size of the capillary bed? This explanation may be correct as has often been suggested but it is worth emphasizing that we have not found a marked discrepancy between the changes in pulmonary capacity and chest size.

Comparison of the expansion of the chest in the sitting and recumbent positions is interesting. We will consider first the diaphragmatic excursion. While recumbent there is a very slight increase in the maximum excursion but the difference is less than a centimeter. The movement of the diaphragm during the determination of the reserve air is much decreased. This is evident on both sides although slightly more on the right. While sitting the reserve excursion of the right diaphragm is 5.0 cm and of the left 3.0 cm and it decreases to 0.7 and 1.0 respectively in recumbency. On the other hand the "complementary" diaphragmatic excursion (during a forced inspiration starting from the mid capacity) shows a very striking increase in recumbency.

The measurements of the areas of the lungs on a radiograph at different respiratory positions (at maximum expiration and inspiration, and after a normal expiration and inspiration) indicate the changes in the size of these areas during the determination of the several subdivisions of the total volume (vital capacity, reserve air, complementary and tidal air). By comparing these differences in area in the sitting and recumbent positions we find that in the latter posture there is relatively a small variation in

SUMMARY AND CONCLUSIONS

The effects of posture upon pulmonary capacity and the size and expansion of the chest have been observed in 10 healthy males by comparing measurements made in the recumbent and sitting postures.

It has been found that when recumbent there are slight decreases in the total volume, the vital capacity and the residual air but the reserve air decreases markedly. On the other hand there is a marked increase in the volume of the complementary air.

Similar and parallel decreases although proportionally less marked have been demonstrated to occur in the size of the chest. This diminution is most marked at mid capacity and it is caused by an upward displacement of the diaphragm.

An analysis of the expansion of the chest in both sitting and recumbent positions shows also parallel changes. In the latter posture the diaphragmatic excursion and the change in the area of the projection of the lungs corresponding to the reserve air are considerably reduced while the reverse is true in relation to the complementary air.

These observations furnish additional data regarding the close correlation existing between the pulmonary capacity and the size and expansion of the chest and indicate the necessity for adopting a standard posture when investigations of this nature are made.

BIBLIOGRAPHY

- 1 Anthony, A. J., *Deutsches Arch. f. klin. Med.*, 1930 *clviii*, 129. Untersuchungen über Lungenvolumen und Lungenventilation.
- 2 Binger, C. A. L. and Brown, G. R. *J. Exper. Med.*, 1924 *xxxix*, 677. Studies on the Respiratory Mechanism of Lobar Pneumonia. A Study of Lung Volume in Relation to the Clinical Course of the Disease.
- 3 Bohr, C., *Deutsches Arch. f. klin. Med.* 1907 *lxxxviii*, 385. Die funktionellen Änderungen in der Mittellage und Vitalkapazität der Lungen.
- 4 Briscoe, J. C., *Quart. J. Med.* 1919-20, *vi*, 293. The Mechanism of Postoperative Massive Collapse of the Lungs.
Idem, *Lancet* 1931 *ii*, 513. The Mechanism of Inflation of the Lungs.
- 5 Calhoun, A., Cullen, G. E., Harrison, T. R., Wilkins, W. L. and Tims, M. M. *J. Clin. Invest.* 1931 *v*, 833. Studies in Congestive Heart Failure. XIV. Orthopnea: Its Relation to Ventilatory Vital Capacity, Oxygen Saturation and Acid Base Condition of Arterial and Jugular Blood.
- 6 Christie, C. D. and Beames, A. J. *Arch. Int. Med.* 1922 *xxx*, 34. The Estimation of Normal Vital Capacity with Especial Reference to the Effect of Posture.
- 7 Hamilton, W. F. and Morgan, A. B. *Am. J. Physiol.* 1931-32 *xviii*, 526. Mechanism of the Postural Reduction in Vital Capacity in Relation to Orthopnea and Storage of Blood in the Lungs.
- 8 Hurtado, A. and Fray, W. W. *J. Clin. Invest.* 1933 *xii*. Studies of Total Pulmonary Capacity and Its Subdivisions. II. Correlation with Physical and Radiological Measurements.

- 9 Livingstone J L, *Lancet* 1928 i 754 Variations in the Volume of the Chest with Changes in Posture
- 10 Plesch C *Ztschr f exper Path u Therap* 1913 xiii, 165 Die pathologische Physiologie des Lungenvolumens und seine Beziehung zum Kreislauf
- 11 Wilson, W H *J Physiol*, 1927-28 lxxv 54 The Influence of Posture on the Volume of the Reserve Air

STUDIES OF TOTAL PULMONARY CAPACITY AND ITS SUBDIVISIONS IV PRELIMINARY OBSERVATIONS ON CASES OF PULMONARY EMPHYSEMA AND OF PNEUMOCONIOSIS

By ALBERTO HURTADO¹ WALTER W. FRAY AND WM. S. McCANN

(From the Department of Medicine of the School of Medicine and Dentistry of the University of Rochester and the Medical Clinic of the Strong Memorial Hospital Rochester New York)

(Received for publication May 15 1933)

There has been no practical method of estimating in a quantitative way the degree of respiratory inefficiency in cases of chronic pulmonary disease. Although many observations have been made in regard to their underlying pathological anatomy and physiology there has not been any serious attempt to correlate such observations with the clinical condition especially with respect to adaptation to the increased respiratory demands on physical activity. Investigators have usually been concerned with circulatory and internal respiratory functions. In previous papers (3) (4) we have presented the results of our measurements of capacity of the lungs and of chest expansion in an attempt to establish the normal variation thus enabling one to distinguish pathological deviations. In the present communication are presented similar observations in cases of chronic pulmonary diseases.

METHODS AND CASES STUDIED

A complete description of the methods used in measuring the total pulmonary air and its subdivisions and in the prediction of their normal values in a given case has been discussed in previous papers (3) (4). Measurements of pulmonary ventilation during physical activity have also been made using a modified bicycle ergometer and a 100 liter Tissot spirometer adapted to the graphic registration of the respirations. In some cases arterial blood was obtained and its oxygen saturation and carbon dioxide content determined by the manometric method of Van Slyke (7). We have studied 15 cases, 9 cases of pulmonary emphysema and 6 cases of pneumoconiosis. Several patients presented evidence of heart disease but no evidence of heart failure was present at the time of these observations. In each group there was considerable variation in the extent of pulmonary disease. This was an important consideration as one of the main purposes of this investigation was to correlate the findings with the degree of functional respiratory disability. A summary of each case is given below.

¹ Travelling Fellow of the Rockefeller Foundation

- Case 1 Male 41 years Chronic cough for 27 years Severe asthmatic attacks during the last few years *Very severe dyspnea on physical activity* No cardiac symptoms
Chest markedly emphysematous Heart normal Radiograph showed increased linear markings and emphysema
Diagnosis Chronic bronchitis Asthma Pulmonary emphysema
- Case 2 Male 54 years Chronic cough and asthmatic attacks for 20 years *Severe dyspnea on physical activity* No cardiac symptoms
Chest markedly emphysematous Heart normal Electrocardiogram normal Radiograph showed increased linear markings
Diagnosis Chronic bronchitis Asthma Pulmonary emphysema
- Case 3 Male 51 years Chronic cough expectoration and asthmatic attacks for two years *Severe dyspnea on physical activity*
No cardiac symptoms
Chest very emphysematous Radiograph showed slight left sided cardiac enlargement and increased lung markings Electrocardiogram presented evidence of myocardial damage
Diagnosis Chronic bronchitis Asthma Pulmonary emphysema Chronic myocarditis (compensated)
- Case 4 Male 41 years Chronic cough and asthmatic attacks for 18 years *Only slight dyspnea on physical activity* No cardiac symptoms
Chest not emphysematous in appearance Hyperresonance on percussion over precordial and hepatic areas Electrocardiogram normal Radiograph of the chest showed prominence of the lung marking and of the pulmonary artery suggesting early fibrosis and pulmonary arteriosclerosis
Diagnosis Chronic bronchitis Asthma Pulmonary emphysema Pulmonic arteriosclerosis
- Case 5 Male 60 years Dry cough for many years *Moderate dyspnea on physical activity* Frequent attacks of precordial pain for two or three years
Chest definitely emphysematous in appearance Heart enlarged to the left General arteriosclerosis
Diagnosis Arteriosclerotic heart disease Pulmonary emphysema
- Case 6 Male 26 years Asthmatic attacks since childhood *No dyspnea on physical activity*
Chest not emphysematous Heart normal Radiograph of chest showed increased linear markings and evidence of an early emphysema
Diagnosis Asthma Pulmonary emphysema
- Case 7 Male 41 years Attacks of precordial pain for seven years During the last two years attacks of unconsciousness accompanied with convulsions *No dyspnea on exertion*
Chest markedly emphysematous Heart enlarged to the left Radiograph of the lungs showed pulmonary fibrosis and emphysema
Diagnosis Coronary heart disease Question of Adams-Stokes syndrome Pulmonary emphysema
- Case 8 Male 50 years *Slight dyspnea on exertion*
Chest does not appear emphysematous Heart enlarged to the left Electrocardiogram showed left ventricular preponderance and myocardial damage Radiograph of the chest suggested pulmonary emphysema pleural thickening and cardiac hypertrophy
Diagnosis Arterio-sclerotic heart disease Pulmonary emphysema

- Case 9* Male 61 years Chronic cough for three years *Very severe dyspnea* on physical activity No cardiac symptoms
Chest markedly emphysematous Heart enlarged to the left Electrocardiogram showed an intraventricular conduction defect Radiograph of the chest revealed a marked degree of emphysema enlargement of the left ventricle and increased linear markings
Diagnosis Chronic bronchitis Arteriosclerotic heart disease Pulmonary emphysema
- Case 10* Male 48 years Had worked in sand blasting for 6 years Chronic cough for four years *Severe dyspnea on physical activity* No cardiac symptoms
Chest emphysematous Heart not enlarged Electrocardiogram gave questionable evidence of myocardial damage Radiograph of the chest showed marked increase in linear markings and typical appearance of pneumoconiosis and emphysema at both bases
Diagnosis Pneumoconiosis Pulmonary fibrosis
- Case 11* Male 35 years Had worked at sand blasting for 5 years Chronic cough for two years *Moderate dyspnea on exertion* Able to carry on light work Complaints of palpitation on physical activity
Chest did not appear emphysematous Heart not enlarged Radiograph of the chest showed characteristic changes of pneumoconiosis pulmonary fibrosis and emphysema Electrocardiogram normal
Diagnosis Pneumoconiosis Pulmonary fibrosis
- Case 12* Male 46 years Had worked at sand blasting for 2 years Slight cough during the last year *Moderate dyspnea on physical activity* No cardiac symptoms
Chest had normal appearance Heart not enlarged Electrocardiogram normal Radiograph of the chest suggested pneumoconiosis and pulmonary emphysema
Diagnosis Pneumoconiosis Pulmonary fibrosis
- Case 13* Male 42 years Had been exposed to inhalation of siliceous dust for 18 months *Moderate dyspnea on exertion* No other symptoms
Chest had emphysematous appearance Heart slightly enlarged to the left Electrocardiogram indicated left ventricular preponderance Radiograph of the chest showed changes consistent with pneumoconiosis (Early second stage)
Diagnosis Pneumoconiosis Pulmonary fibrosis Myocardial disease (compensated)
- Case 14* Male 42 years Had worked at sand blasting for 8 months Chronic cough during the last two years He thought that in the last two months he had had *slight dyspnea on physical activity* No other symptoms
Chest normal Heart not enlarged Radiograph of the chest showed increase in size of the hilum and increased markings but no mottling Appearance of emphysema in both bases
Diagnosis Question of pneumoconiosis Question of pulmonary fibrosis
- Case 15* Male 56 years Had been severely exposed to inhalation of siliceous dust 7 years Hemoptysis frequent cough and *severe dyspnea on exertion* Edema of lower extremities
Chest markedly emphysematous Heart greatly enlarged Electrocardiogram showed right ventricular preponderance Radiograph of

the lungs revealed a diffuse soft mottling Pulmonary artery prominent The total volume of the blood was increased
 Diagnosis Pneumoconiosis Pulmonary fibrosis Sclerosis of the pulmonary artery Myocardial disease Secondary polycythemia

Observations on pulmonary capacity

Pulmonary emphysema The calculated and the observed values for the total pulmonary air and its main subdivisions in the nine subjects having pulmonary emphysema are presented in Table 1, and are shown in Figure 1 In these cases the total capacity of the lungs which was ob-

TABLE 1

Comparison of observed pulmonary capacity with calculated normal values in emphysema and in pneumoconiosis

Case number	Total capacity			Vital capacity			Mid capacity			Residual air		
	Calculated	Observed	Difference	Calculated	Observed	Difference	Calculated	Observed	Difference	Calculated	Observed	Difference
	liters	liters	per cent	liters	liters	per cent	liters	liters	per cent	liters	liters	per cent
Pulmonary emphysema												
1	8.53	6.64	-22.2	6.66	3.05	-54.2	3.24	4.67	+44.1	1.89	3.59	+90.0
2	7.25	8.52	+17.5	5.66	2.70	-52.3	2.75	6.56	+138.5	1.59	5.82	+266.0
3	5.62	5.96	+6.0	4.39	1.42	-67.7	2.13	5.32	+149.0	1.23	4.54	+269.1
4	6.89	7.59	+10.1	5.38	4.95	-8.0	2.62	3.72	+41.9	1.51	2.64	+74.8
5	6.47	6.18	-4.5	5.05	4.00	-20.8	2.46	3.56	+44.7	1.42	2.18	+53.5
6	5.02	5.76	+14.7	3.92	3.64	-7.2	1.91	2.96	+54.9	1.10	2.12	+92.7
	6.20	5.08	-18.1	4.84	3.60	-25.7	2.36	1.96	-17.0	1.36	1.48	+8.8
	5.70	4.32	-24.3	4.45	2.98	-33.1	2.17	1.66	-23.5	1.25	1.34	+7.2
	5.98	5.04	-15.8	4.67	1.44	-69.2	2.27	4.08	+79.7	1.31	3.60	+174.8

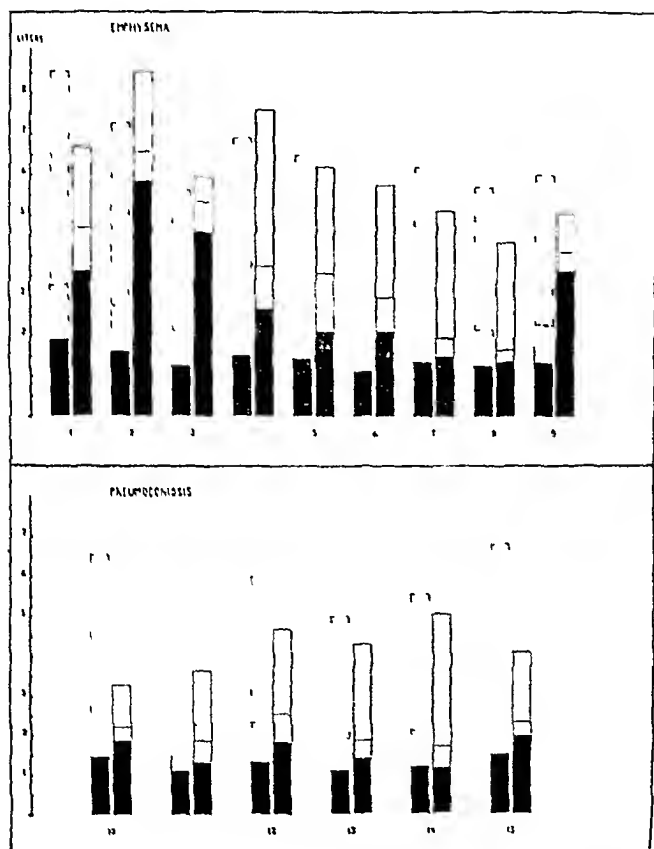


FIG. 1. CALCULATED AND OBSERVED PULMONARY CAPACITY IN CASES OF PULMONARY EMPHYSEMA AND PNEUMOCONIOSIS.

Each case is represented by two columns. The one on the left with broken lines is the predicted value and the right column is the observed volume.

In black residual air. Transverse line dividing the white area (vital capacity) is the mid capacity level.

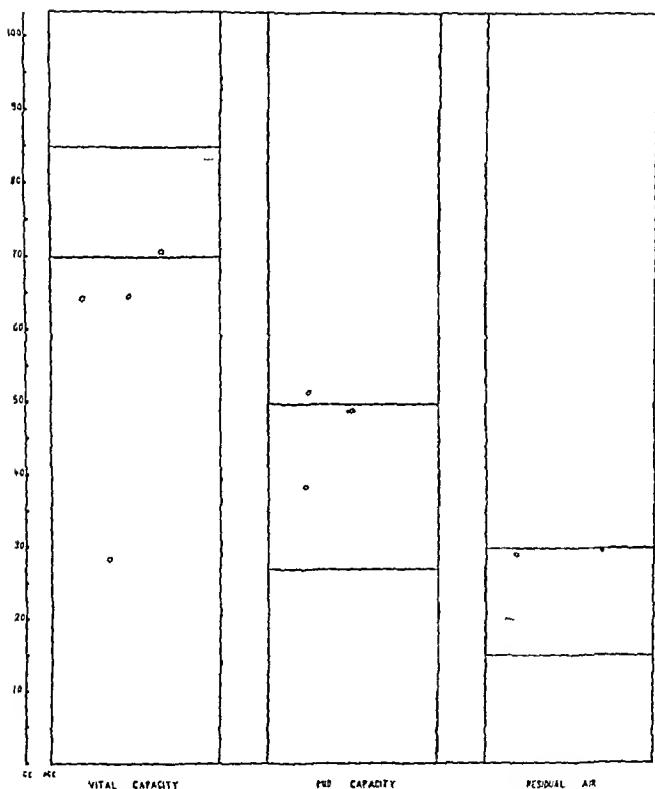


FIG 2 RELATIVE PULMONARY CAPACITIES (TOTAL CAPACITY = 100 PER CENT) IN CASES OF PULMONARY EMPHYSEMA AND PNEUMOCONIOSIS

The shaded areas represent limits of normal variations. Black dots are cases of pulmonary emphysema. Circles are cases of pneumoconiosis.

These alterations in the absolute values of the pulmonary airs are reflected in the relative values. That this is so may be appreciated by a study of Table 2. The vital capacity is reduced and the residual air cor-

TABLE 2

Relative values of subdivisions of pulmonary capacity in emphysema and pneumoconiosis

Case number	Ratio Vital capacity Total volume $\times 100$		Ratio Residual air Total volume $\times 100$		Ratio Mid capacity Total volume $\times 100$		Ratio Complementary air Vital capacity $\times 100$	
	Normal	Observed	Normal	Observed	Normal	Observed	Normal	Observed
Pulmonary emphysema								
1	78.0	45.9	22.0	54.1	38.0	70.4	79.4	64.6
2	78.0	31.7	22.0	68.3	38.0	77.0	79.4	72.6
3	78.0	23.8	22.0	76.4	38.0	87.6	79.4	45.1
4	78.0	65.2	22.0	34.8	38.0	49.1	79.4	78.2
5	78.0	64.7	22.0	35.3	38.0	57.6	79.4	65.5
6	78.0	63.2	22.0	36.8	38.0	51.4	79.4	77.0
7	78.0	70.8	22.0	29.1	38.0	38.5	79.4	88.1
8	78.0	64.3	22.0	35.7	38.0	38.7	79.4	89.3
9	78.0	28.5	22.0	71.4	38.0	80.9	79.4	66.6
Pneumoconiosis								
10	78.0	43.6	22.0	56.4	38.0	67.3	79.4	75.0
11	78.0	63.7	22.0	36.0	38.0	50.9	79.4	76.7
12	78.0	61.5	22.0	38.4	38.0	54.4	79.4	79.7
13	78.0	69.3	22.0	30.7	38.0	43.4	79.4	54.5
14	78.0	77.1	22.0	22.9	38.0	33.6	79.4	86.1
15	78.0	51.4	22.0	48.6	38.0	56.5	79.4	84.6

respondingly increased. In severe cases the residual air occupies a much greater proportion of the total volume than the vital capacity. A profound alteration in the ability to ventilate the alveoli has accordingly taken place.

In many cases, especially in the severe ones, the complementary air makes up a smaller portion of the vital capacity than is the case with normal persons. This defect is a noteworthy feature in Cases 1 and 3, in which the volume of reserve air is strictly normal. It appears therefore that the distended lungs cannot be deflated to the normal level, but that a forced expiration expels nevertheless a normal quantity of air owing to the change in volume of mid capacity.

When the vital capacity is composed chiefly of reserve air, or when the latter volume is not as markedly reduced as the complementary air, there is probably a further handicap to effective alveolar ventilation.

Christie (2) found the volume of the reserve air was smaller after a maximum inspiration than is the case when it is measured after a normal expiration and interpreted this as an indication of diminished elasticity of

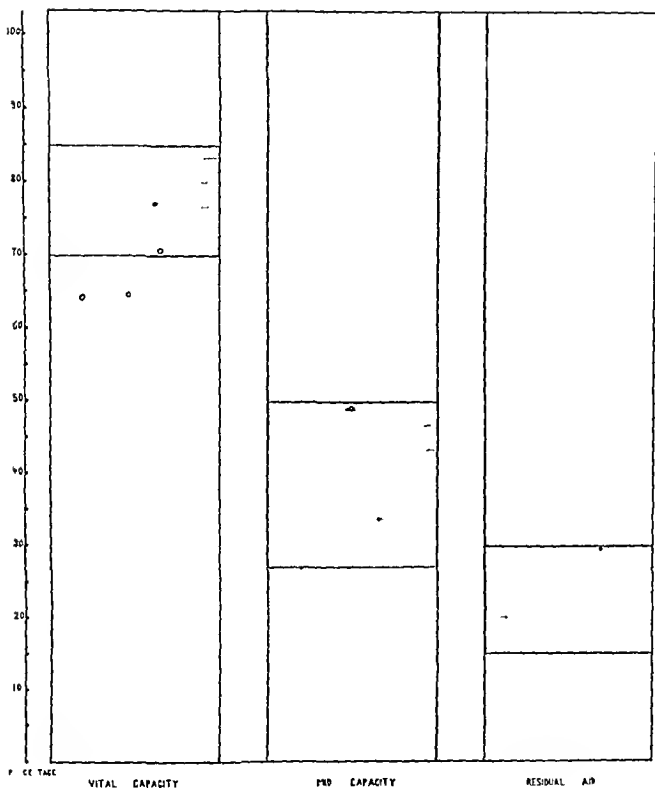


FIG 2 RELATIVE PULMONARY CAPACITIES (TOTAL CAPACITY = 100 PER CENT) IN CASES OF PULMONARY EMPHYSEMA AND PNEUMOCONIOSIS

The shaded areas represent limits of normal variations. Black dots are cases of pulmonary emphysema; circles are cases of pneumoconiosis.

the lungs in pulmonary emphysema. There is apparently a decrease in the ability of individuals to deflate the lungs after they have been fully expanded. We have investigated this phenomenon in all our cases and have found that it is not always present. It occurred in two instances but was not evident in some of the most severe and most unquestionable cases of emphysema.

Pneumoconiosis. The results of our observations in this condition differ from those obtained in pulmonary emphysema. The observed total capacity of the lungs is as a rule lower than the calculated. This decrease is proportional to the clinical and radiological evidence of the severity of the disease. The lungs of these patients contain at maximum inspiration a smaller volume of air than would be predicted from the radiographic measurements of the chest. The diminution in total capacity is caused entirely by decrease in the vital capacity. The residual air is moderately increased in almost all cases and this change indicates that there is some degree of pulmonary emphysema associated with the fibrotic changes. This observation agrees with the frequent demonstration by pathologists and radiologists of emphysematous areas in the lungs of these patients. The mid capacity is found to be normal in most instances. The vital capacity is reduced in proportion to the total volume, and the residual air greater but these alterations are relatively less than in cases of pulmonary emphysema. The increase in the ratio (Residual air/Total volume) is chiefly due to diminution in the vital capacity. The two components of the vital capacity, the reserve and complementary air, show a normal relationship to each other in pneumoconiosis.

Chest expansion

Changes in the ratio (Area at maximum expiration/Area at maximum inspiration) $\times 100$ (measured on the doubly exposed radiograph of the lungs) are especially interesting. From previous studies (4) of normal male subjects we concluded that a ratio higher than 72.0 may be considered as indicating diminished expansion. In all but two of the cases of pulmonary emphysema this ratio was higher than 72 (Table 3). In these two the diagnosis of emphysema was made on clinical grounds only. In neither of them did the pulmonary capacity show marked significant abnormality. It appears then that in undoubted cases of pulmonary emphysema there is a conspicuous decrease in ability to expand the chest (see Figure 3). The diaphragmatic excursion and the lateral expansion of the chest also are as a rule decreased. The angle of movement of a rib in changing from full expiration to maximum inspiration was markedly reduced in the majority of patients. In two of the severest cases movement was almost nil.

In pneumoconiosis the ratio (Area at maximum expiration/Area at maximum inspiration) $\times 100$ was within upper normal limits in all but two cases. In one it was markedly altered, due to reduced expansion of

TABLE 3
Chest expansion

Case number	Ratio Area at maximum expiration Area at maximum inspiration $\times 100$	Dia- phragmatic excursion *	Lateral expansion	Rib movement	Circum- ference expansion
		cm	cm	degrees	cm
Pulmonary emphysema					
1	90.9	0.3	2.3	20	4.0
2	86.8	3.5	2.2	7	4.5
3	84.9	2.4	0.4	3	2.0
4	77.9	5.8	2.8	14	10.0
5	70.7	5.1	1.0	11	8.0
6	75.5	4.3	2.2	22	7.0
7	77.4	3.3	1.0	12	8.0
8	66.9	4.7	0.6	10	5.0
9	82.9	4.8	0.2	2	3.0
Pneumoconiosis					
10	89.5	0.8	1.8	8	4.0
11	70.0	5.0	2.5	15	6.0
12	73.5	4.7	0.9	13	5.0
13	60.6	5.3	2.8	18	4.0
14	70.9	4.1	2.3		6.0
15	73.8	5.3	1.1	3	3.5

* Average of both diaphragms

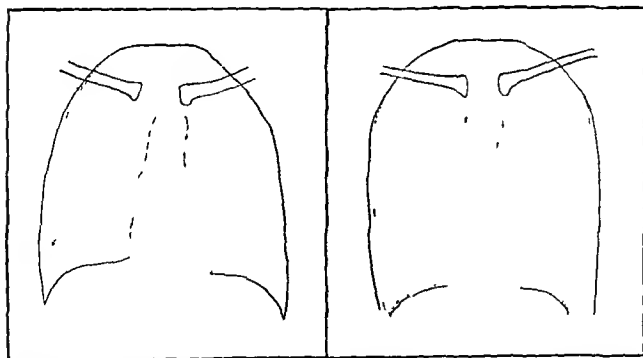


FIG 3 OUTLINE OF THE LUNG FIELDS OF MAXIMUM INSPIRATION AND EXPIRATION IN A NORMAL MAN (LEFT) AND IN A CASE OF PULMONARY EMPHYSEMA (RIGHT)

the chest. The diaphragmatic excursion, the lateral expansion of the chest and the movement of the rib were usually within normal limits. Reduction in chest expansion is not characteristic of pneumoconiosis and pulmonary fibrosis, as it is of pulmonary emphysema.

Relationship of dyspnea to alterations in capacity of the lungs

A striking correlation is found to exist between the observed alterations in capacity of the lungs and the tendency to dyspnea. In all those cases in which there was severe limitation in physical activity we find marked changes in the absolute and relative pulmonary capacity. A high ratio (Residual air/Total volume) is almost always accompanied by a pronounced degree of dyspnea on physical exertion. On the other hand in Cases 4, 5, 7, 8 and 14 in which such a ratio was either normal or but slightly increased the history of dyspnea on exertion was not conspicuous. The tendency to dyspnea in the cases of pneumoconiosis was less severe as a rule than in those with pulmonary emphysema and the changes in the relative pulmonary capacities were correspondingly not as great. In two cases in which the ratio of residual air to total volume was high dyspnea on physical activity was, however, also severe.

While discussing the adaptation to physical activity it will be pertinent to present some observations on the measurement of pulmonary ventilation during exercise. The observations included graphic registration of the number of respirations, tidal and minute volumes, of patient while engaged in work in a specially designed chair ergometer. These investigations are summarized graphically in Figure 4. Normally the process of adaptation to physical activity necessitates greater frequency of respiration as well as increase in the tidal volume so that the ventilation per minute is considerably increased. Peabody (6) found that efficient ventilation during exercise is closely related to the vital capacity and that when this is low the maximum possible pulmonary ventilation is reduced proportionally. In pneumoconiosis in which there is a decrease in the vital capacity patients were able to increase the number as well as the depth of the respirations during exercise yet the total ventilation was considerably less than in men. In pulmonary emphysema an abnormal response to physical exercise is evident. The vital capacity was low and there was diminished ability to expand the chest. Emphysematous individuals during physical activity tend to increase the rate rather than the depth of breathing (Fig 4). Difficulty in expansion of the chest in emphysema is so great that when accessory muscles are brought into play voluntarily increase in the depth of breathing is accompanied by decrease in rate. In all the cases subjected to exercise tests there has been severe dyspnea on exertion and in each case the maximum ventilation attainable has been less than normal.

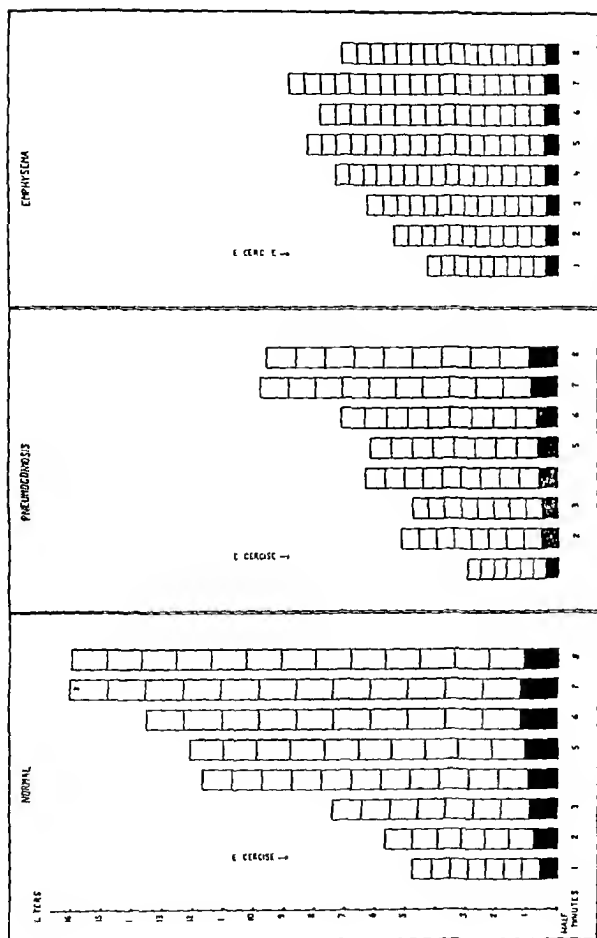


FIG. 4 VENTILATION DURING EXERCISE. DIAGRAM CONSTRUCTED FROM SEVERAL OBSERVATIONS MADE ON NORMAL MAN INDIVIDUALS AND OF CASES OF PNEUMONIOSIS AND PULMONARY EMPHYSEMA.

Height of each column represents the total ventilation during succeeding half minutes. Each subdivision in the column represents the tidal volume and the figure within the column the number of respirations during that half minute.

Pulmonary capacity and arterial saturation with oxygen

In seven cases the arterial blood has been examined to determine its content of carbon dioxide and the degree of saturation with oxygen. The results and the corresponding pulmonary capacities are presented in Table 4. The lowest values for saturation of arterial blood with oxygen have been found in cases in which the ratio (Residual air/Total volume) was abnormally high.

TABLE 4
Relationship of arterial oxygen saturation to pulmonary capacity

Cases	Oxygen saturation arterial blood	CO content arterial blood volume	Decrease in vital capacity	Increase in residual air	Ratio Residual air Total volume
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
(3)	72.9	49.3	-67.7	+269.1	76.2
(1)	79.6	47.6	-54.2	+90.0	54.1
(2)	80.0	46.7	-52.3	+266.0	68.3
(10)	80.6	55.2	-71.9	+29.1	56.1
(11)	81.2	46.2	-41.9	+16.9	36.3
(4)	95.6	50.2	-8.0	+74.8	34.8
(12)	95.7	44.2	-39.5	+34.5	35.5

DISCUSSION

Marked and significant changes in the absolute and relative pulmonary capacities exist as these studies show in cases of pulmonary emphysema. The results are in agreement with the investigations of Lundsgaard and Schierbeck (5) and Anthony (1), who found a normal total volume but a marked decrease in the vital capacity and a corresponding increase in the residual air. The fact that there are definite alterations in the capacity of the lungs in cases of this nature, and that these changes are closely correlated with the clinical condition and the tendency to dyspnea, is of considerable importance. The diagnosis of pulmonary emphysema is not so easy to establish clinically as it appears to be. An emphysematous appearance of the chest may be due entirely to skeletal changes without alteration in the lungs and conversely actual emphysema in the pathological-physiological sense of the word, may be present and may escape clinical detection because the chest is of normal size and shape. Not infrequently symptoms of respiratory failure are attributed to heart disease especially in people past middle age when pulmonary changes alone are responsible for the symptoms. In doubtful cases the measurement of the pulmonary capacity will aid in establishing the correct diagnosis. The calculated pulmonary capacity in some cases of pulmonary emphysema is not strictly to be regarded as 'normal' since we are using as a basis for prediction the size of the chest which in itself may be pathological. To be normal the capacity of the lungs in a given case must correspond to the volume of

the chest before pathological alterations occurred. We have investigated in normal men the possible correlation of thoracic volume with other bodily characteristics, and have not found any correlation by means of which we could predict normal values from other data such as height, weight, or surface area. In emphysema observed total pulmonary capacities agree fairly closely with the size of the chest estimated from measurements of radiographs. The most significant alterations occur in the relative values of the subdivisions of total capacity.

There are no previous observations in the literature regarding pulmonary capacity in cases of pneumoconiosis and pulmonary fibrosis. We have observed that there are significant changes in such cases, the degree of which is closely correlated with the functional efficiency of respiration and with the anatomical changes revealed in the radiological examinations. From these observations it seems probable that the determination of the pulmonary capacity may be of value in the proper evaluation of the degree of respiratory disability. In Case 14 in which the evidence of fibrosis in the lungs was small the pulmonary capacity was normal. But in Cases 10 and 11 in which it was great the capacity of the lungs was much diminished. In four of five cases there was moderate increase in the volume of residual air due probably to secondary emphysema usually found in association with advanced pulmonary fibrosis.

SUMMARY AND CONCLUSIONS

- 1 In seven of nine cases of pulmonary emphysema the total pulmonary capacity observed corresponded closely with that predicted from measurements of the chest cavity. In two it was slightly less. Increase in the volume of the residual air and a corresponding reduction in the vital capacity was observed in all cases.

- 2 In emphysematous patients there was definite reduction in expansion of the chest, the degree being closely correlated with alterations in the relative pulmonary capacities.

- 3 In cases of pneumoconiosis (pulmonary fibrosis) the total capacity of the lungs observed was less than that predicted from measurements of the chest due to decrease in the vital capacity. The residual air was moderately increased in four of five cases. In one the changes were minimal.

- 4 Decrease in expansion of the chest was not a significant feature of cases of pneumoconiosis (pulmonary fibrosis).

- 5 Cases in which the ratio (Residual air/Total volume) was abnormally high were found to exhibit low saturation of the arterial blood with oxygen indicating poor alveolar ventilation.

Preliminary observations on response to exercise showed that the capacity to ventilate the lungs was limited in a severe case of emphysema and in one of pneumoconiosis compared with that in a normal man.

Further observations will be necessary to establish a relation between the degree of functional disability and abnormalities in pulmonary capacity.

BIBLIOGRAPHY

- 1 Anthony, A. J. *Deutsches Arch. f. klin. Med.*, 1930 cxvii, 129. Untersuchungen über Lungenvolumina und Lungenventilation.
- 2 Christie R. V., *J. Clin. Invest.*, 1932 xi, 1099. The Lung Volume and its Subdivisions. I. Methods of Measurement.
- 3 Hurtado A. and Boller C. *J. Clin. Invest.* 1933 xii. Studies of Total Pulmonary Capacity and its Subdivisions. I. Normal Absolute and Relative Values.
- 4 Hurtado, A. and Gray, W. W., *J. Clin. Invest.* 1933 xii. Studies of Total Pulmonary Capacity and its Subdivisions. II. Correlation with Physical and Radiologic Measurements.
- 5 Lundsgrård C., and Schierbeck K., *Proc. Soc. Exper. Biol. and Med.* 1922-23 xx, 165. Studies on Lung Volume. IX. Patients with Lung Emphysema Pulmonum.
- 6 Perbody F. W. *Am. J. Med. Sc.* 1915 cx, 100. Cardiac Dyspnea.
- 7 Peters J. P. and Van Slyke D. D. *Quantitative clinical chemistry*. II. Methods. Williams & Wilkins Co. Baltimore 1932.

THE EFFECT OF DIGITALIS ON THE VENOUS PRESSURE OF NORMAL INDIVIDUALS

By DAVID A. RYLAND

(From the Department of Medicine Stanford University School of Medicine
San Francisco)

(Received for publication April 17 1933)

That the administration of digitalis in heart failure causes an increase in cardiac output (per minute and per beat) and a fall in venous pressure is well known (1). It is also well established that the exhibition of digitalis to normal men and dogs results in a decreased output (2 3 4 5 6 7) but data on venous pressure in normal individuals following digitalis are inconclusive.

In animals Villaret Grellety Bosviel and coworkers (8) found a fall of venous pressure after intravenous injection of ouabain Kaufmann (9) Popper (10), and Dock and Trinter (5 6) found a decreased venous pressure following intravenous injection of strophanthin and digitalis. Plummer (11) noted increased venous pressure after strophanthin and digitoxin. Yokota (12) found a small decrease greater decrease and increase in venous pressure with intravenous injection of small medium and large doses respectively of strophanthin. Capps and Matthews (13) found no change after digitalis.

In man Stewart and Cohn (7) found a marked fall in venous pressure in one normal subject after oral administration of digitin using the Moritz Tribora (20) technique of venous pressure determination. Simultaneously in the first subject and in two other subjects they found no constant change when a less accurate method was used (that of allowing blood to rise in a dry tube).

As Eyster (14) pointed out it is impossible to determine whether the changes produced by digitalis in output are cardiac or extra cardiac in origin unless the changes in venous pressure are known. In decompensated hearts it is agreed (5 7) that the increased output is due largely to the change in ventricular tone any peripheral action of digitalis being relatively unimportant (although Wollheim (15) and others (16 17) credit the reduction in effective (systemic) blood volume with more importance in relieving congestive failure).

Opinion differs widely however regarding the origin of the digitalis effects in the normal organism. Cohn and his coworkers (7 18 19) unable to demonstrate venous pressure changes hold that the decreased cardiac output is due to diminished ventricular filling caused by increased tone

of the heart muscle. Dock and Tainter (5, 6) on the other hand believe that the decreased output is due to diminished ventricular filling caused by lowered venous pressure (with lessened return flow of blood to the heart), which they have shown to be the result of pooling of blood in the portal system.

Grollman (1) states that it is impossible at present to say definitely if the primary cause of the observed decrease in the cardiac output following administration of digitalis to normal individuals is due to its action on the heart or on peripheral structures. This study was designed to obtain data on the height of venous pressure before and after the administration of full doses of digitalis to normal human beings to determine whether the question of cardiac or extra-cardiac action of the drug in decreasing output could be answered.

METHODS

Thirty-seven control and 82 experimental observations were made on venous pressure before and after the administration of digitalis (in single doses) in 9 trials on 8 normal subjects.

Apparatus and technique. The method used was one of the direct (venepuncture) types, a simplification of the Moritz-Libora (20) technique as suggested by Dock. The system consisted of an intravenous needle and a manometer (a straight 20 cm. length of glass tubing 3 mm. inside diameter), connected by means of a Kaufmann-Luer syringe. The needle and syringe were boiled before each determination, while the manometer was kept in 95 per cent alcohol.

Before use, the system was connected and filled with sterile physiological saline to avoid the misleading effects of the viscosity of a blood column rising in a dry tube. The saline-filled system was suspended over the antecubital fossa of the subject and venepuncture performed in the usual aseptic manner. The plunger of the syringe was then withdrawn until the manometer was in direct communication with the vein, and fastened in place. The column of saline was allowed to fall in the manometer until equilibrium was reached when the height of the top of the column above the vein was read. Eight millimeters (found by trial) was subtracted from each reading to allow for capillarity.

Each reading was made with the subject in the supine position on a thin mattress without sag, so that the olecranon and the dorsum of the thorax were on the same plane. To avoid the effects of exercise (21) the subjects assumed this position 20 to 30 minutes before each determination. No readings were taken immediately after the subjects had eaten. Room temperature was approximately constant.

All clothing above the waist was removed. Stasis for venepuncture was momentary only. After each reading the upper arm was compressed to insure patency of the needle and to allow the saline to rise and fall again for a confirmatory reading. Because of the number of venepunctures in each subject both arms were used, preliminary observations having shown that this proceeding introduced no error.

No effort was made to approximate the level of the antecubital vein to that of the right auricle, as we were more interested in following changes from hour to hour in any given subject and felt that accuracy would be sacrificed if attempts were made to obtain absolute venous pressure (22).

Subjects There were 8 subjects 6 men and 2 women. Age varied from 35 to 63 years. Two series of observations were made on one woman. All subjects were ambulatory patients on the neuro-psychiatry ward diagnosed psychoneurosis, psychasthenia, involutional melancholia and schizophrenia. Most of them cooperated nicely. None showed any evidence of cardiovascular abnormalities; in particular there were absolutely no signs or symptoms of cardiac decompensation in any subject. None of these patients experienced the symptoms of pain, lassitude or dyspnea noted by Stewart and Cohn (7) after digitalis administration.

Digitalis The digitalis powder used was kindly supplied and standardized by Doctor A. B. Stockton. It assayed 108 mgm to one Hatcher cat unit (predicted clinical dose = 21 to 35 mgm per kgm) and 25 mgm to one pigeon emetic dose (predicted clinical dose = 25 mgm per kgm). The dosage varied from 1.0 to 1.6 gram (19.2 to 30.0 mgm per kgm). Each subject received the powder in one dose at six o'clock in the morning before breakfast.

RESULTS

The initial venous pressure determinations in five of the subjects were obviously fallacious, being 2.2, 4.1 and 4.9 cm higher than the average of the remaining control observations in three cases and 1.0 and 3.1 cm lower in two. In another instance a single reading 6.8 cm higher was found. All of these erroneous values were discarded in the computations of results and do not appear in the figures or tables; the net result of discarding these observations is to minimize slightly the venous pressure decrease to be pointed out below.

There are 37 control observations distributed over 9 series. The variabilities ($100\sigma/\text{Mean}$) of the series are 14.1, 11.7, 11.4, 10.1, 7.3, 7.1, 6.4, 4.2 and 3.2 per cent respectively. The average variability is 8.4 per cent. There are 82 observations after digitalis.

An afternoon rise of venous pressure was noted in 4 of 6 series of control observations. The mean afternoon rise in these 6 series was 0.4 cm of saline. This is in the same direction but of much less degree than the diurnal variation noted with an indirect method by Hooker (23). No correction for this small variation was made in the computations.

The control and experimental results are summarized in Table 1 (hours approximated), Table 2 and Figure 1. It is evident that digitalis caused a fall of venous pressure of greatest degree (minus 1.96 cm saline) at 24 to 32 hours after administration with a return to the control level at 72 to 96 hours.

DISCUSSION

In Figure 2 (data in Table 3) is represented a comparison of our results on venous pressure (A) with those of Cohn and Stewart (4, 7) on cardiac output in humans (B) and in dogs (C). All means are computed by averaging observations about their respective modes.

TABLE 1
Effect of giving digitalis on venous pressure in normal individuals

Subjects*	Controls†	Hours after oral administration of digitalis‡															
		1	8	10	12	16	28	36	52	60	9†	102	132	156	180	192	
Hu ♂ 62.1 kgm 1.2 kgm	11.2 11.8 11.0 10.5 Ncm = 11.1 Pulsc = 60	-1.5 87		+2.0 83			+1.4 87			+1.7 90							
		-1.9 106			-0.3 95		-1.1 85										
		-0.1 107	+3.0 107	+0.3 90		-0.2 99	-0.3 100				-1.0 88	+1.3 96	+2.0 91			-1.6 101	
Oc ♂ 73.0 kgm 1.5 kgm	13.6 13.5 11.5 13.1 Ncm = 13.7 Pulsc = 75																
		-2.6 90		+2.7 95													
		-2.5 91		-2.5 83		+2.0 99	-1.5 91	-1.0 99		+1.5 96							
Jp ♂ 60.0 kgm 1.3 kgm	10.7 12.5 11.2 11.7 11.5 Ncm = 13.3 Pulsc = 73	-0.7 110		-0.1 115	-1.2 112	-0.8 101	-4.1 112	-5.5 99	-3.2 101	-0.1 101			-0.9 96	+0.1 101			
		-1.0 91	-0.1 91	+0.0 99	+0.1 105	-0.1 91	-2.6 94	-3.1 91	-3.1 84	-2.7 71	-1.9 79						
Lo ♂ 57.3 kgm 1.6 kgm	11.0 12.1 10.5 12.3 Ncm = 11.5 Pulsc = 69	-1.0 110		-2.0 90			-0.7 107		-3.2 96	+0.9 101	-0.8 104	+0.0 101					
		+0.6 111	-3.1 91	-3.1 101	-3.1 108	-1.3 113	-3.0 113			-0.6 103	-0.5 117	+0.5 111		-0.6 113	-1.1 99	-1.5 111	
Mi ♀ 10.0 kgm 1.2 kgm	8.2 10.7 8.1 9.6 Ncm = 9.2 Pulsc = 87	-2.6 87	-3.6 93	-2.2 92		-1.5 97	-2.7 110	-1.1 99	-0.7 101	-1.8 106	-0.1 110	+1.0 110	-0.2 103	-1.5 103	+1.1 99	-0.0 101	

* Subjects arranged in order of increasing magnitude of digitalis dose per kilogram body weight

† Under controls are given all venous pressure observations in centimeters of saline, with their means the means of the control pulses (rds per minute) are also given

‡ Figures in upper line preceded by (+) or (-) are deviations from control mean venous pressure in centimeters of saline. Lower line figures are pulse rates in percentage of control mean

TABLE 2

Effect of giving digitalis on the venous pressure of normal individuals Means of all observations

Hours after administration of digitalis	Range of hours after digitalis	Number of observations	Venous pressure. Deviation from control mean	Pulse rate Percentage of control mean
<i>hours</i>	<i>hours</i>	<i>number</i>	<i>cm saline</i>	<i>per cent</i>
9.3	4-17	34	-1.35	97.0
31.7	28-38	14	-1.96	98.5
57.3	52-62	12	-1.08	95.6
88.9	76-104	10	+0.06	102.5
167.8	120-270	12	-0.28	102.1
		82		
4.8	4-5	10	-2.26	98.3
9.2	7-10	13	-0.70	93.6
13.5	11-17	11	-1.29	99.7
		34		

In the lower part of the table, the observations in the first 17 hours are further separated. The comparatively high venous pressure in the 7-10 hour range is due to the slow pulse (93.6 per cent). See discussion in text.

Direction of changes Digitalis, in normal individuals, causes a decrease in both cardiac output and venous pressure. It is axiomatic that a cardiac cause for diminished output will result in an increased venous pres-

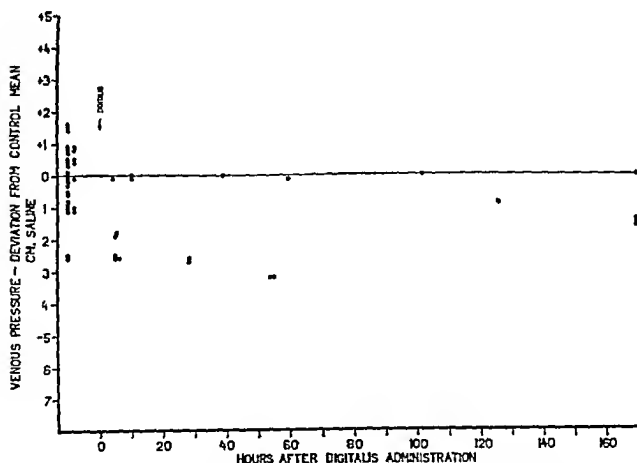


FIG 1 SCATTER DIAGRAM SHOWING ALL OBSERVATIONS

sure, and that an extra-cardiac production of lowered venous pressure will bring about a decreased cardiac output

Duration of changes The time relations of venous pressure changes to cardiac output changes are similar. The lowest mean venous pressure

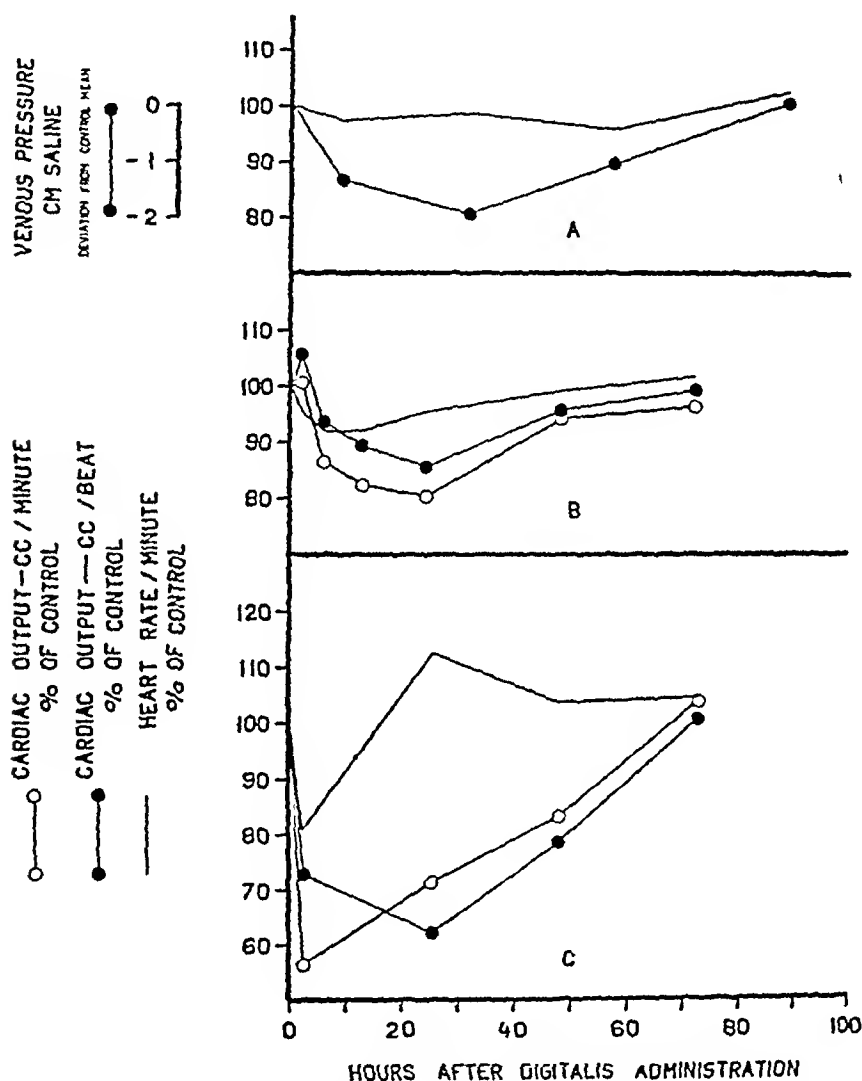


FIG 2 THE EFFECT OF DIGITALIS ON VENOUS PRESSURE AND CARDIAC OUTPUT IN NORMAL INDIVIDUALS

A Venous pressure B Output in human beings (7) C Output in dogs (4)

occurred at 31.7 hours, while the minute and stroke volume in man fell to their lowest values at 24 hours (after oral administration of digitalis). The 8 hour difference is probably due to the paucity of observations and to the necessity of averaging observations about their modes. In dogs, be-

TABLE 3

The effects of digitalis administration on venous pressure and on cardiac output A comparison

Hours after digitalis administration	Number of observations	Venous pressure Deviation from control mean	Stroke volume Percentage of control	Minute volume Percentage of control	Pulse rate Percentage of control
hours	number	cm saline	per cent	per cent	per cent
<i>A Venous pressure in the normal human being</i>					
9 3	34	-1 35			97 0
31 7	14	-1 96			98 5
57 3	12	-1 08			95 6
88 9	10	+0 06			102 5
<i>B Cardiac output in the normal human being (7)</i>					
2	2		105 5	100 5	95 5
6	10		93 4	86 4	92 5
13	8		89 3	82 1	92 2
24	7		85 4	80 1	95 4
48	6		95 6	95 2	99 1
72	4		99 0	96 2	101 5
<i>C Cardiac output in the normal dog (4)</i>					
2 5	11		72 9	56 5	80 9
25 5	12		62 2	71 2	112 5
48	3		78 4	83 0	103 7
73	4		100 5	103 8	104 5

cause of the intravenous administration of the drug, time relationships are not strictly comparable, even here, however, the stroke volume is lowest at 25 5 hours

Venous pressure returned to the control level between 72 and 96 hours after digitalis. Cardiac output in man reached normal values in 72 hours (stroke volume 99 0 and minute volume 96 2 per cent of their respective controls). Cardiac output in dogs was slightly above the control in 73 hours. (The apparent 16 day duration of depressed output in Stewart and Cohn's (7) Subject 1 is undoubtedly due to a high control reading for the values at 4, 5, 7, and 16 days after digitalis are remarkably constant—87, 89, 88, and 82 per cent of the control minute volume respectively.)

The effect of digitalis on the A-V conduction time and T wave of the electrocardiogram disappears in 5 to 22 days (24 25). We made no electrocardiographic studies, but with the doses used the calculated duration of conduction time and T wave effects in our series would be 8 to 14 days (26).

The duration of digitalis action on both cardiac output and venous pressure is more fleeting, then, than on the central T wave and A-V con-

sure, and that an extra-cardiac production of lowered venous pressure will bring about a decreased cardiac output

Duration of changes The time relations of venous pressure changes to cardiac output changes are similar. The lowest mean venous pressure

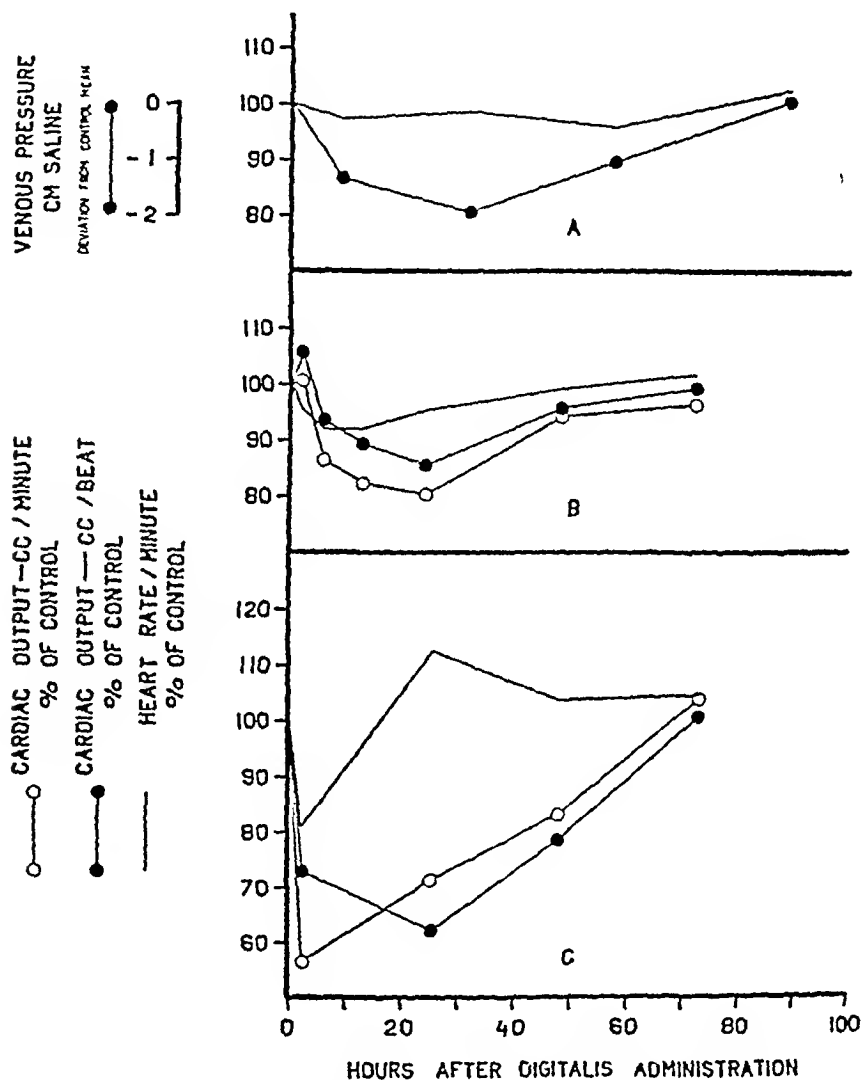


FIG 2 THE EFFECT OF DIGITALIS ON VENOUS PRESSURE AND CARDIAC OUTPUT IN NORMAL INDIVIDUALS

A Venous pressure B Output in human beings (7) C Output in dogs (4)

occurred at 31.7 hours, while the minute and stroke volume in man fell to their lowest values at 24 hours (after oral administration of digitalis). The 8 hour difference is probably due to the paucity of observations and to the necessity of averaging observations about their modes. In dogs, be-

be regarded as constant, and the effects on diastolic size, as measures of the effect of digitalis on tone." Obviously, heart work is a function of the output per beat and is not, as Cohn and Steele suggest, measured by the oxygen consumption of the entire organism. Starling and Visscher (29) have demonstrated that diastolic volume varies with heart work, so that the decreased size of the heart observed by Cohn and Stewart (4, 7) should occur as a result of the decreased output. In expelling equal quantities of blood, a small heart must make greater excursions than does a larger heart. This fact amply explains the observations of Cohn and Stewart (4) without the necessity of invoking changes of tone. The effect is great enough to exist despite the decreased volume which the small heart ejects after digitalis.

Cohn and Steele (18) further state "the decrease in volume output which has been noticed was thought to result from the tonic effect of digitalis upon the heart (4), in consequence of which its size diminished so much, that a large or even a usual volume of blood could not be received and, therefore, could not be ejected." If this were the case, venous pressure should rise as it does in cardiac tamponade (27), we have demonstrated on the contrary, that venous pressure actually falls. Moreover, Wiggers and Stimson (30) showed by intraventricular pressure curves that the heart under digitalis offers no more resistance to inflowing blood than does the normal undigitalized heart.

Finally, Tainter (31) was able to reproduce the typical digitalis effects of decreased output and lowered venous pressure in cats with the Gibbs artificial heart apparatus in which preparation the heart is replaced by a mechanical pump and in which, therefore there could be absolutely no question of changes in "heart tone."

Relation of venous pressure to pulse rate If digitalis bradycardia were due to the Bainbridge reflex, following decreased auricular pressure, we should expect the greatest slowing of pulse to occur in those subjects with the largest fall of venous pressure. However, Figure 3 (data in Tables 2 and 4) shows that the opposite is the case (the increased venous pressure in Figure 3 C is due to errors of selection because of the small number of observations).

The fact that the pulse rate mean chanced not to fall below 95 per cent of the control in our series in no way invalidates the significance of the venous pressure decrease, for in the dogs of Cohn and Stewart (4), the lowest outputs were recorded with a mean pulse rate of 112.5 per cent of the control. Burwell, Neighbors, and Regen (3) also noted that output returned toward normal when their subjects experienced nausea or had more rapid pulses. In one patient with acute hepatitis (not included in this series) in whom digitalis produced partial heart block (pulse rate 41 to 47) and intense nausea and vomiting of four days duration the venous pressure fell only 1.4 and 0.8 cm. at 5 and 11 hours respectively. The ef-

duction time changes It is more prolonged than the rise of arterial pressure in dogs

Degree of changes Dock and Tainter (5) found in their dogs F4 and F5 that falls in venous pressure of 3.5 and 6.5 cm. of water corresponded to minute output reductions of 41 and 55 per cent of controls, respectively. On the average, 1.0 cm. of venous pressure was equivalent to 10.1 per cent of minute volume. Beck and Isaac (27) found, in experimental cardiac tamponade studies, that a 3.3 cm. rise in venous pressure was associated with a 36 per cent reduction in cardiac output, and a 10.0 cm. rise with a 64 per cent reduction. Thus, 1.0 cm. of venous pressure was equivalent to 8.6 per cent of minute volume. Wiggers (28) presents a figure showing increasing systolic discharge in parallel with experimentally increased venous pressure (heart rate constant). Measurements taken from this figure permit the calculation that 1.0 cm. of venous pressure is equivalent to 10.87 per cent of stroke volume.

Figure 2, in which the venous pressures of one group of subjects are compared with the cardiac outputs of a similarly studied group, shows that at the time of the maximal digitalis effect in man the venous pressure had fallen 1.96 cm. and the minute and stroke volumes 19.9 and 14.6 per cent of their respective controls; that is, a 1.0 cm. venous pressure change corresponded to a 10.15 and a 7.45 per cent change in minute and stroke volumes.

From a comparison of our results with those of Cohn and Stewart (4, 7), it is evident that venous pressure and cardiac output changes in normal individuals after digitalis correlate remarkably well in direction, time, and degree. The observations on direction can be explained only by a peripheral mechanism of digitalis action, those on time support such a mechanism, for the change in venous pressure and cardiac output (like that in arterial pressure in dogs) is less prolonged than the effect on the T wave and conduction time, the degree of the venous pressure change is sufficient to explain the observed fall of cardiac output. The correlation which Stewart and Cohn (7) make between output and cardiac area is not so well marked as that between output and venous pressure, and neither supports nor controverts either the cardiac or extra-cardiac hypothesis of digitalis action.

The question of cardiac tone According to Cohn and Steele (18), digitalis decreases cardiac output in normal cardiovascular systems by an increase in cardiac tone; the only evidence presented that the tone is increased is that of Cohn and Stewart (4). The latter observers found, radiographically, that the hearts of normal dogs became smaller and exhibited greater ventricular excursions after digitalis.

Cohn and Steele (18) state "since, in the experiments of Cohn and Stewart (4), the measurements were made during periods when the dogs were in so-called basal states, the work which their hearts performed may

be regarded as constant, and the effects on diastolic size, as measures of the effect of digitalis on tone." Obviously, heart work is a function of the output per beat and is not, as Cohn and Steele suggest, measured by the oxygen consumption of the entire organism. Starling and Visscher (29) have demonstrated that diastolic volume varies with heart work, so that the decreased size of the heart observed by Cohn and Stewart (4, 7) should occur as a result of the decreased output. In expelling equal quantities of blood, a small heart must make greater excursions than does a larger heart. This fact amply explains the observations of Cohn and Stewart (4) without the necessity of invoking changes of tone. The effect is great enough to exist despite the decreased volume which the small heart ejects after digitalis.

Cohn and Steele (18) further state "the decrease in volume output which has been noticed was thought to result from the tonic effect of digitalis upon the heart (4), in consequence of which its size diminished so much that a large or even a usual volume of blood could not be received and, therefore, could not be ejected." If this were the case, venous pressure should rise as it does in cardiac tamponade (27), we have demonstrated, on the contrary, that venous pressure actually falls. Moreover, Wiggers and Stimson (30) showed by intraventricular pressure curves that the heart under digitalis offers no more resistance to inflowing blood than does the normal undigitalized heart.

Finally, Tainter (31) was able to reproduce the typical digitalis effects of decreased output and lowered venous pressure in cats with the Gibbs artificial heart apparatus, in which preparation the heart is replaced by a mechanical pump and in which, therefore, there could be absolutely no question of changes in 'heart tone'.

Relation of venous pressure to pulse rate If digitalis bradycardia were due to the Bainbridge reflex, following decreased auricular pressure, we should expect the greatest slowing of pulse to occur in those subjects with the largest fall of venous pressure. However, Figure 3 (data in Tables 2 and 4) shows that the opposite is the case (the increased venous pressure in Figure 3 C is due to errors of selection because of the small number of observations).

The fact that the pulse rate mean chanced not to fall below 95 per cent of the control in our series in no way invalidates the significance of the venous pressure decrease, for in the dogs of Cohn and Stewart (4), the lowest outputs were recorded with a mean pulse rate of 112.5 per cent of the control. Burwell, Neighbors, and Regen (3) also noted that output returned toward normal when their subjects experienced nausea or had more rapid pulses. In one patient with acute hepatitis (not included in this series) in whom digitalis produced partial heart block (pulse rate 41 to 47) and intense nausea and vomiting of four days duration the venous pressure fell only 1.4 and 0.8 cm. at 5 and 11 hours respectively. The ef-

fect of digitalis in reducing venous pressure is powerful enough, then, to overcome the counteracting effect of digitalis bradycardia (which, unopposed, would increase auricular pressure)

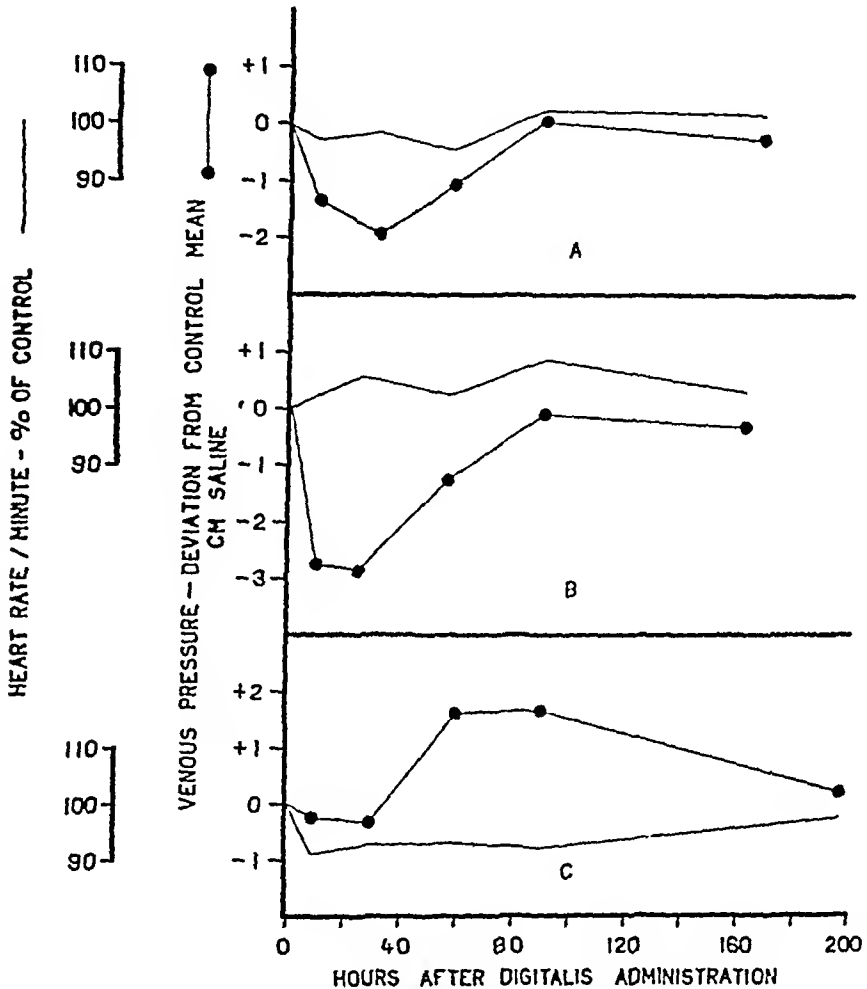


FIG 3 THE RELATION OF PULSE RATE TO VENOUS PRESSURE

A Means of all observations B Means of those with least slowing of pulse C Means of those with greatest slowing of pulse (In C the apparent rise of venous pressure is due to the small number of observations between 40 and 120 hours after giving digitalis)

The production of decreased venous pressure The hypothesis advanced by Dock and Tainter (5, 6, 31) to account for the lowered venous pressure and cardiac output after digitalis is that this drug constricts the hepatic veins, resulting in diminished return flow through the inferior vena cava. References to earlier workers on this "sluice mechanism" are given in their papers, along with their corroboratory experimental data. The

TABLE 4
Relation of venous pressure to pulse rate

Hours after digitalis administration	Number of observations	Venous pressure Deviation from control mean	Pulse rate Percentage of control
hours	number	cm. saline	per cent
<i>A Means of all subjects</i>			
9.3	34	-1.35	97.0
31.7	14	-1.96	98.5
57.3	12	-1.08	95.6
88.9	10	+0.06	102.5
167.8	12	-0.28	102.1
<i>B Means of subjects with least slowing of pulse in the first 36 hours</i>			
9.4	15	-2.75	102.4
25.6	7	-2.88	105.8
56.3	7	-1.24	102.3
88.9	7	-0.11	108.7
161.8	10	-0.38	103.0
<i>C Means of subjects with greatest slowing of pulse in the first 36 hours</i>			
9.0	14	-0.24	91.0
30.7	4	-0.33	92.7
60.5	2	+1.60	93.0
89.2	2	+1.65	92.0
197.6	2	+0.20	97.5

hypothesis is confirmed by the results of other workers (15, 16, 17). Cohn and Steele (18) state that "in order to be effective in shutting off from the volume of blood returning to the right auricle as much as a quarter, the lumina of the two vessels" [right and left hepatic veins] "must be reduced to zero—must be completely obliterated." However, Blalock and Bradburn (32) produced a 75 per cent reduction in cardiac output by intestinal trauma and associated altered circulatory state in the portal area, Elman and Cole (33) found that approximately 60 per cent of the total blood volume accumulates in the splanchnic area within a short time after portal vein ligation, Enderlen and coworkers (34) found that death occurs from reduced arterial pressure and cardiac output in 10 to 60 minutes after portal vein occlusion. With such dramatic results following the prevention of portal outflow, it is easily possible that digitalis should lower cardiac output only 20 per cent and venous pressure 2 cm. by constricting hepatic vein radicles.

The anatomic evidence for the "sluice mechanism" has been investigated in cats, dogs, and human beings by Elias and Feller (36). Popper

(37), and Bauer, Dale, Poulsson, and Richards (35), the subject is reviewed by the latter. In brief, the findings suggest that in the dog the mechanism is brought about by the anatomic construction at the caval openings of the hepatic veins, in the cat and in man the hepatic vein radicles probably play a more important role. Whether the greater sensitivity of the dog to digitalis action on cardiac output (Fig 2) may be attributed to the anatomic findings is not known.

Clinical applications The reduction in heart size noted by Stewart and Cohn (7) is undoubtedly due to the digitalis effects studied above, such action, if sustained, would retard cardiac hypertrophy. In a recent paper, Christian (38) proposes continuous complete digitalization of patients with enlarged hearts (and of those in whom cardiac enlargement may be expected) while compensated, under the clinical impression that enlargement and decompensation are retarded. Because of the importance of this subject, the impression should be confirmed or disproved by experiments designed to determine how long the digitalis effect on heart size, venous pressure, and cardiac output can be sustained in animals, normal human beings, and those compensated patients whom Christian suggests digitalizing to retard heart failure.

CONCLUSIONS

1 Digitalis causes, in normal human beings and dogs, a decreased cardiac output and a decreased venous pressure. The greatest effect occurs at about 24 to 32 hours after administration of the drug, with a return to normal levels in 72 to 96 hours.

2 The observed changes support the hypothesis that digitalis owes its action to a peripheral effect, probably on the hepatic vein radicles, in reducing the return flow of blood to the heart. The hypothesis that the digitalis action follows changes in cardiac tone is negatived by all available data.

3 Digitalis bradycardia is not due to the fall of venous pressure. On the contrary, the slowing of the heart, by causing the normal increase in venous pressure, partially conceals the effect of digitalis in reducing the return flow of blood to the heart.

The author takes this opportunity to thank Dr William Dock for assistance in the preparation of this paper.

BIBLIOGRAPHY

- 1 Grollman, A, *The Cardiac Output of Man in Health and Disease* Charles C Thomas, Springfield and Baltimore, 1932, p 177
- 2 Harrison, T R., and Leonard, B W, *J Clin Invest*, 1926, *iii*, 1 *The Effect of Digitalis on the Cardiac Output of Dogs and Its Bearing on the Action of the Drug in Heart Disease*
- 3 Burwell, C S., Neighbors, De W., and Regen, E M, *J Clin Invest*, 1928, *v*, 125 *The Effect of Digitalis upon the Output of the Heart in Normal Man*

- 4 Cohn, A E and Stewart, H J, *J Clin Invest* 1928 vi, 53 The Relation Between Cardiac Size and Cardiac Output per Minute Following the Administration of Digitalis in Normal Dogs
- 5 Dock W and Tainter M L, *J Clin Invest*, 1929-30 viii, 467 The Circulatory Changes after Full Therapeutic Doses of Digitalis, with a Critical Discussion of Views on Cardiac Output
- 6 Tainter, M L, and Dock W *J Clin Invest*, 1929-30, viii, 485 Further Observations on the Circulatory Actions of Digitalis and Strophanthus with Special Reference to the Liver and Comparisons with Histamine and Epinephrine
- 7 Stewart H J, and Cohn A E, *J Clin Invest*, 1932, xi 917 Studies on the Effect of the Action of Digitalis on the Output of Blood from the Heart. III Part 1 The Effect on the Output in Normal Human Hearts Part 2 The Effect on the Output of Hearts in Heart Failure with Congestion, in Human Beings
- 8 Villaret, M Saint Giron, F, and Justin Besançon, L *La Pression Veineuse Peripherique* Masson Paris, 1930, p 278
- 9 Kaufmann M *Rev de med*, 1884 iv, 381 Effets physiologiques de la digitaline amorphe
- 10 Popper J *Ztschr f klin Med*, 1889, xvi, 97 Ueber die physiologische Wirkung des Strophantins
- 11 Plumier, L, *Arch internat de Physiol*, 1909, viii, 1 Etude expérimentale des variations de la pression veineuse
- 12 Yokota M *Tohoku J Exper Med*, 1923, iv, 23 Über die Wirkung der Arzneimittel auf den Blutdruck besonders den venösen
- 13 Capps J A and Matthews, S A, *J A M A*, 1913, lxi, 388 Venous Blood Pressure as Influenced by the Drugs Employed in Cardiovascular Therapy
- 14 Eyster, J A E *Tr A Am Physicians* 1928 xliii, 17 (In discussion on paper of Cohn and Stewart)
- 15 Wollheim E *Klin Wchnschr*, 1928, vii, 1261 Kompensation und Dekompensation des Kreislaufs
- 16 Schürmeyer, A (Quoted by Mies (17))
- 17 Mies H, *Verhandl d deutsch Gesellsch f Kreislaufforsch*, 1931, p 208 Ueber die Wirkung des Strophanthin auf die zirkulierende Blutmenge
- 18 Cohn, A E and Steele J M *J Clin Invest*. 1932 xi, 871 Studies on the Effect of the Action of Digitalis on the Output of Blood from the Heart I The Effect on the Output of the Dog's Heart in Heart Lung Preparations
- 19 Stewart H J and Cohn, A E *J Clin Invest*, 1932, xi 897 Studies on the Effect of the Action of Digitalis on the Output of Blood from the Heart II The Effect on the Output of the Hearts of Dogs Subject to Artificial Auricular Fibrillation
- 20 Moritz, F, and Tabora D, *Deutsches Arch f klin Med*, 1921 xcvi, 475 Über eine Methode beim Menschen den Druck in oberflächlichen Venen exakt zu bestimmen
- 21 White, H L *Am J Physiol*, 1924, lxi, 410 Circulatory Responses to Exercise in Man and their Bearing on the Question of Diastolic Heart Tone
- 22 Evans W *New Eng J Med* 1932 ccvii 934 Venous Pressure
- 23 Hooker D R *Am J Physiol* 1914, xxvi, 73 Observations on the Venous Blood Pressure in Man

- 24 Cohn, A E, Fraser, F R, and Jamieson R A, *J Exper Med* 1915, *xxi*, 593 The Influence of Digitalis on the T Wave of the Human Electrocardiogram
- 25 White, P D, and Sattler, R R, *J Exper Med*, 1916 *xxiii*, 613 The Effect of Digitalis on the Normal Human Electrocardiogram, with Especial Reference to A-V Conduction
- 26 Bromer, A W, and Blumgart H L, *J A M A*, 1929, *xcii*, 201 The Maintenance Dose of Digitalis An Electrocardiographic Study
- 27 Beck, C S, and Isaac, L, *J Thoracic Surg*, 1931, *i*, 124 Pneumocardiac Tamponade A Study of the Effects of Atmospheric Pressure, Negative Pressure and Positive Pressure upon the Heart
- 28 Wiggers, C J, *Modern Aspects of the Circulation in Health and Disease* Lea and Febiger, Philadelphia and New York, 1923, 2d ed., p 107, fig 31
- 29 Starling, E H, and Visscher, M B, *J Physiol*, 1927, *lxii*, 243 The Regulation of the Energy Output of the Heart
- 30 Wiggers, C J, and Stimson, B, *J Pharmacol and Exper Therap*, 1927, *xxx*, 251 Studies on the Cardiodynamic Action of Drugs III The Mechanism of Cardiac Stimulation by Digitalis and g-Strophanthin
- 31 Tainter, M L, *Arch internat de Pharmacodyn et de therap*, 1932, *xlii*, 186 Use of the Gibbs Artificial Heart in the Study of Circulatory Phenomena, with Descriptions of Improvements in the Device and of Responses to Some Drugs
- 32 Blalock, A, and Bradburn, H, *Arch Surg*, 1930, *xx*, 26 Distribution of the Blood in Shock
- 33 Elman, R, and Cole, W H, *Proc Soc Exper Biol and Med*, 1932, *xxix*, 1122 Loss of Blood as a Factor in Death from Acute Portal Obstruction
- 34 Enderlen, Hotz, and Magnus-Alsleben, *Ztschr f d ges exper Med*, 1914, *iii*, 223 Die Pathologie und Therapie des Pfortaderverschlusses
- 35 Bauer, W, Dale, H H, Poulsson, L T, and Richards, D W, *J Physiol*, 1932, *lxxiv*, 343 The Control of Circulation through the Liver
- 36 Ehas, H, and Feller, A *Stauungstypen bei Kreislaufstorungen* Springer, Wien u Berlin, 1926
- 37 Popper, H, *Klin Wehnschr*, 1931, *x*, 1693, 2129 Ueber Drosselvorrichtungen an Lebervenen
- 38 Christian, H A, *J A M A*, 1933, *c*, 789 The Use of Digitalis other than in the Treatment of Cardiac Decompensation

A COMPARISON OF THE CREATININE AND UREA CLEARANCE TESTS OF KIDNEY FUNCTION

By J M HAYMAN JR J A HALSTED AND L E SEYLER

(From the H K Cushing Laboratory of Experimental Medicine in the Department of Medicine of Western Reserve University and the Medical Service of Lakeside Hospital Cleveland)

(Received for publication May 1 1933)

The urea clearance test developed by Möller, McIntosh and Van Slyke (1) has one disadvantage, namely, that maximum and standard clearances are only comparable by reference to the average normal value for each or by multiplying the standard clearance by a constant. In addition the standard clearance, involving the square root of the urine volume is difficult to interpret. The creatinine clearance test, developed by Rehberg (2), is not subject to these objections, since the excretion of creatinine is independent of urine volume. It therefore seemed worth while to compare the creatinine and urea clearances as a test of renal function, to determine if there was sufficient practical advantage in the creatinine test to compensate for the added technical difficulties.

Rehberg presented evidence that led him to believe that the creatinine clearance represented the volume of glomerular filtrate and on this assumption calculated the quantities of urea and chloride reabsorbed. Rehberg's conception has been accepted by Wyschegorzewa (3), Bergwall (4), and others (5, 6), questioned by Cope (7) and Ekehorn (8), and denied by Shannon, Jolliffe and Smith (9). The evidence at present available does not, in our opinion justify unreservedly accepting the creatinine clearance as equal to the volume of glomerular filtrate, and while this lessens the value of the test as a tool in the study of renal physiology it need not detract from its usefulness as a practical test of kidney function.

METHODS

Hospital patients were kept in bed for the period of the test. Dispensary patients and normal subjects were allowed to sit in a chair or engage in light laboratory work. Some tests were made after fourteen hours fast the majority after a light breakfast which MacKay (10) found had no effect on the urea clearance. Three to five grams of creatinine were given by mouth an hour to one and one-half hours before the beginning of the test. At the beginning of the test the bladder was emptied as completely as possible and a sample of venous blood obtained. Approximately an hour later, but timed to the nearest minute, the bladder

was again emptied as completely as possible and a second sample of blood obtained. If there was any doubt whether the subject could empty the bladder, he was catheterized. All blood analyses were made on serum.

Urea nitrogen in urine and serum was estimated by the gasometric method of Van Slyke (11), in a few instances by Van Slyke and Cullen's (12) method. All analyses were made in duplicate. Creatinine in urine and serum was estimated by Rehberg's (13) modification of Folin's method using a colorimeter with Burk optical system.

The ingestion of three to five grams of creatinine increases the plasma concentration to 5 to 10 mgm per cent. This decreases the effect of substances other than creatinine in plasma which give the Jaffe reaction as well as making the estimation more certain. Since Behre and Benedict (14) and Gaebler (15) have doubted the existence of creatinine in normal blood, and since Gaebler could recover only a relatively small fraction of the creatinine added to blood, it was felt that the creatinine analyses had to be examined before any reliance could be placed on the calculation of creatinine clearance by the formula $UV/B = C$. By the method used in these experiments the average recovery of creatinine added to serum in amounts equivalent to 0.5 to 150 mgm per 100 cc was 93.5 per cent in 15 experiments, the extremes being 80 and 124 per cent. Table I shows

TABLE I

Comparison of creatinine clearances calculated from total chromogenic substances and from ingested creatinine only

Name	Before ingestion		After ingestion		Creatinine clearance †	
	Serum creatinine	Creatinine excreted	Average serum creatinine	Creatinine excreted		
	mgm per cent	mgm per minute	mgm per cent	mgm per minute	A	B
J M H	1.27*	1.42*	9.63	12.72	132	134
			8.31	10.30	124	126
			12.22	12.50	102	101
White	0.95	1.37	6.16	10.05	163	166
Wilson	1.08	1.59	8.28	8.08	106	100
Letcher	1.01	.89	9.20	11.05	120	124

* Average of 4 determinations

† A—clearance calculated from total chromogenic substance in serum and urine

B—clearance calculated from ingested creatinine only, subtracting value of chromogenic substances normally present in serum and average rate of excretion of creatinine before ingestion

that there is no difference in the clearance calculated on total chromogenic substances in serum and urine after ingestion of creatinine and in the clearance calculated on ingested creatinine only. Gaebler and Keltch (16) have shown that all the chromogenic material adsorbed on Lloyd's reagent

and released again is indistinguishable from creatinine. Therefore, a clearance calculated on the material released from Lloyd's reagent should be higher than that calculated on total chromogenic substances when no creatinine has been fed, since other substances account for a considerable fraction of the value obtained for "creatinine" in normal serum. After ingestion of creatinine, the clearances calculated before and after the use of Lloyd's reagent should show much less difference. Table II shows that

TABLE II

Comparison of creatinine clearances calculated from total chromogenic substances and from material released from Lloyd's reagent with and without ingestion of creatinine

Experimental number and condition	Urine volume	Total creatinine			Released from Lloyd's		
		Serum	Urine	Clearance	Serum	Urine	Clearance
	<i>cc per minute</i>	<i>mgm per cent</i>	<i>mgm per cent</i>	<i>cc per minute</i>	<i>mgm per cent</i>	<i>mgm per cent</i>	<i>cc per minute</i>
1 No	6.84	1.01	131	89	84	136	111
2 creatinine	7.07	1.30	20	109	96	18	133
3 ingested	7.75	1.32	18	106	87	14	125
4 Creatinine	7.72	9.63	165	132	9.03	159	136
5 ingested	1.95	12.22	642	102	10.99	600	106
6	9.35	6.16	108	164	6.02	101	157

this is the case. These experiments led us to believe that with serum concentrations of creatinine above 5 mgm per cent the creatinine clearance could be calculated on total chromogenic substance present in serum and urine without significant error.

In order to estimate the range of creatinine clearance in normal individuals under conditions of hospital and office practice 59 clearances on 59 apparently healthy individuals were tabulated. In 45 of these, only a single test was made. In 14, from 2 to 21 clearances were determined. For these the first clearance determined was tabulated. The range in these 59 observations was from 87 to 232 cc per minute, the mean 144 cc per minute with a standard deviation of 36 cc. When repeated tests are made of the same individual, there is a similar wide dispersion, but the limits for all clearances which we have obtained from normal individuals are only slightly wider. Nor apparently does any normal individual tend to have a constantly high or low clearance.

For this reason, it seemed proper to include all our observations (130) of normal individuals where no recognized unusual factor, such as administration of a drug or exercise, was present to show the normal chance distribution of clearances. If this be done, the range is from 70 to 238 cc per minute, the mean 148 cc per minute with a standard deviation of 34 cc. A frequency polygram of these observations shows a decided skewness, but not sufficient to make unreasonable the belief that the chances of a single

was again emptied as completely as possible and a second sample of blood obtained. If there was any doubt whether the subject could empty the bladder, he was catheterized. All blood analyses were made on serum.

Urea nitrogen in urine and serum was estimated by the gasometric method of Van Slyke (11), in a few instances by Van Slyke and Cullen's (12) method. All analyses were made in duplicate. Creatinine in urine and serum was estimated by Rehberg's (13) modification of Folin's method, using a colorimeter with Barker optical system.

The ingestion of three to five grams of creatinine increases the plasma concentration to 5 to 10 mgm per cent. This decreases the effect of substances other than creatinine in plasma which give the Jaffe reaction, as well as making the estimation more certain. Since Behre and Benedict (14) and Gaebler (15) have doubted the existence of creatinine in normal blood, and since Gaebler could recover only a relatively small fraction of the creatinine added to blood, it was felt that the creatinine analyses had to be examined before any reliance could be placed on the calculation of creatinine clearance by the formula $UV/B = C$. By the method used in these experiments the average recovery of creatinine added to serum in amounts equivalent to 0.5 to 15.0 mgm per 100 cc was 93.5 per cent in 15 experiments, the extremes being 80 and 124 per cent. Table I shows

TABLE I

Comparison of creatinine clearances calculated from total chromogenic substances and from ingested creatinine only

Name	Before Ingestion		After Ingestion		Creatinine clearance †	
	Serum creatinine	Creatinine excreted	Average serum creatinine	Creatinine excreted	A	B
	mgm per cent	mgm per minute	mgm per cent	mgm per minute		
J. M. H.	1.27*	1.42*	9.63	12.72	132	134
			8.31	10.30	124	126
			12.22	12.50	102	101
White	0.95	1.37	6.16	10.05	163	166
Wilson	1.08	1.59	8.28	8.08	106	100
Letcher	1.01	.89	9.20	11.05	120	124

* Average of 4 determinations

† A—clearance calculated from total chromogenic substance in serum and urine

B—clearance calculated from ingested creatinine only, subtracting value of chromogenic substances normally present in serum and average rate of excretion of creatinine before ingestion

that there is no difference in the clearance calculated on total chromogenic substances in serum and urine after ingestion of creatinine and in the clearance calculated on ingested creatinine only. Gaebler and Kelch (16) have shown that all the chromogenic material adsorbed on Lloyd's reagent

This is a lower normal limit than Holten and Rehberg (19) use. They found the clearance always above 100 cc per minute in normal individuals when tested between 10 and 11 a m. But in 89 clearances reported by Rehberg of himself where experimental conditions were more varied regarding posture and fluid intake, the clearance was 9 times between 80 and 100 cc per minute, and twice between 60 and 80 cc per minute, so that a patient cannot be said to have diminished renal function on the basis of a single creatinine clearance test between 60 and 100 cc per minute.

The maximum urea clearance in 56 observations of 25 normal individuals ranged from 38 to 112 cc. per minute, with an average of 74.68 cc per minute and a standard deviation of 17.57 cc, identical with the average of 75 cc per minute found by Möller, McIntosh and Van Slyke. The range in our subjects is somewhat greater than the extremes of 52.2 and 103.8 cc per minute which they found. The mean standard urea clearance in 39 observations of 26 persons was 51 cc per minute with a standard deviation of 10.11 cc. The range was from 30 to 67 cc per minute. This is the same range found by Möller, McIntosh and Van Slyke (28.3 to 69.3 cc per minute) while the mean is but slightly lower. Thus in the case of normal individuals creatinine and urea clearances have about the same degree of dispersion. The creatinine clearance is always numerically greater than the maximum urea clearance, although when the standard urea clearance is calculated using the square root of urine volume, the numerical value may be greater for urea than for creatinine clearance. In estimating reduction of kidney function we have used 148 cc per minute as the average normal creatinine clearance and 75 cc and 54 cc per minute as the average normal maximum and standard urea clearances.

A comparison of creatinine and urea clearances has been made 116 times on 93 patients with Bright's disease and certain other conditions (Tables III, IV and V). We have followed Addis's classification (20) of Bright's disease but have used the term acute instead of initial hemorrhagic Bright's disease. Patients with degenerative Bright's disease, of whom relatively few were examined, have been grouped with the miscellaneous cases in Table V. We have not had the opportunity of examining any patient in whom the diagnosis of "cryptic" degenerative Bright's disease (pure lipoid nephrosis) seemed proper. Cases 107-624 and 151-532 presented a typical picture at the time of some examinations, but had had hematuria some time previously. Both clearance tests frequently show marked reduction in kidney function before there is any elevation of blood urea nitrogen, decrease in two hour phenolsulphonphthalein output, or fixation of specific gravity. This was shown for the urea clearance by Van Slyke and his associates (21) and for creatinine by Holten and Rehberg (19). We have not found the decrease more constant or more marked in one test than the other. Just as in normal persons, the creatinine clearance is always numerically greater than the maximum urea clearance, the

clearance of a normal adult falling between 80 and 216 are about 24 l (Fig 1)

McIntosh, Möller and Van Slyke (17) found that more constant normal values for urea clearance were obtained if a correction was made for surface area. This seems reasonable for Taylor, Drury and Addis (18) were able to show that kidney weights in rabbits varied in proportion to surface area rather than to body weight. To avoid an error greater than ± 5 per cent in using the formula $UP/B=C$, body size can be neglected only in adults between 164 and 176 cm in height. We have corrected the creatinine clearance values by the factor $(175/A)$ where A is surface area

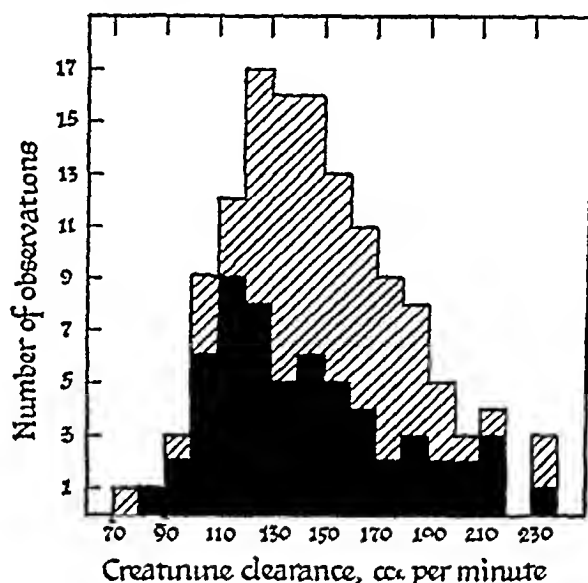


FIG 1 FREQUENCY POLYGRAM OF 130 CREATININE CLEARANCE VALUES ON 59 NORMAL ADULTS

The solid area includes only the first observation made on each person

in square meters. This amounted to more than 10 per cent in 5 instances. The average of the corrected clearances is 145 ± 34 cc per minute, and the dispersion practically the same as for the uncorrected values. Since other unknown factors produce a much greater variation in clearances done on the same individual at different times than the amount of the correction for surface area, we feel that this correction is unnecessary in the case of adults except possibly for those of extreme heights. We have not applied the correction in the patients studied.

From our experience with normal subjects and patients suffering from various diseases, we have come to believe that creatinine clearances below 60 cc per minute (41 per cent of average normal) are definitely abnormal, and those between 60 and 80 cc per minute (41 to 54 per cent average normal) doubtful but significant if a repeated test is in the same range.

This is a lower normal limit than Holten and Rehberg (19) use. They found the clearance always above 100 cc per minute in normal individuals when tested between 10 and 11 a.m. But in 89 clearances reported by Rehberg of himself where experimental conditions were more varied regarding posture and fluid intake, the clearance was 9 times between 80 and 100 cc per minute, and twice between 60 and 80 cc per minute, so that a patient cannot be said to have diminished renal function on the basis of a single creatinine clearance test between 60 and 100 cc per minute.

The maximum urea clearance in 56 observations of 25 normal individuals ranged from 38 to 112 cc per minute, with an average of 74.68 cc per minute, and a standard deviation of 17.57 cc., identical with the average of 75 cc per minute found by Möller, McIntosh and Van Slyke. The range in our subjects is somewhat greater than the extremes of 52.2 and 103.8 cc per minute which they found. The mean standard urea clearance in 39 observations of 26 persons was 51 cc per minute with a standard deviation of 10.11 cc. The range was from 30 to 67 cc. per minute. This is the same range found by Möller, McIntosh and Van Slyke (28.3 to 69.3 cc per minute) while the mean is but slightly lower. Thus in the case of normal individuals creatinine and urea clearances have about the same degree of dispersion. The creatinine clearance is always numerically greater than the maximum urea clearance, although when the standard urea clearance is calculated using the square root of urine volume the numerical value may be greater for urea than for creatinine clearance. In estimating reduction of kidney function we have used 148 cc per minute as the average normal creatinine clearance and 75 cc. and 54 cc per minute as the average normal maximum and standard urea clearances.

A comparison of creatinine and urea clearances has been made 116 times on 93 patients with Bright's disease and certain other conditions (Tables III, IV and V). We have followed Addison's classification (20) of Bright's disease but have used the term acute instead of initial hemorrhagic Bright's disease. Patients with degenerative Bright's disease, of

TABLE III
Creatinine and urea clearances in patients with hemorrhagic Bright's disease

Hospital number	Sex	Age years	Date	Stage of disease	Blood pressure mm Hg	Phenol- sulphon- phthalein per cent in 2 hours	Blood urea nitrogen mgm per cent	Urea clearance cc per minute	Per cent of normal	Creatinine clearance cc per minute	Per cent of normal	Date of death
116-625	F	14	May 17, 1932	Acute	110/70	80	16.5	32.8	44	55	37	
116-212	M	33	January 28, 1932	Acute	110/100		33.6	51.0	68	91	61	
116-555	M	20	January 26, 1933	Acute	135/80	55	10.0	32.8*	61	83	56	
118-311	M	55	October 26, 1932	Acute	110/80	30	25.3	33.3	44	80	51	
150-822	F	27	February 23, 1933	Acute	115/80		9.8	92.5	123	166	112	
119-831	M	12	June 25, 1931	Acute	190/120	20	34.9	12.7*	24	39	26	
			August 27, 1931	Latent	110/90	70	14.0	21.9*	41	69	47	
			December 22, 1932	Latent	120/80		18.0	36.9	49	88	59	
			January 5, 1933	Latent			9.7	31.2	16	73	19	
			January 19, 1933	Latent			21.7	43.2	58	95	61	
			January 23, 1933	Latent			31.1	22.3*	41	66	15	
			January 30, 1933	Latent			9.2	27.8	37	73	49	
150-571	M	38	January 4, 1933	Chronic active	180/100	68	18.2	21.4	29	41	28	
116-216	M	52	April 26, 1932	Chronic active	130/80	30	26.6	16.6*	31	86	58	
107-621	M	15	January 12, 1932	Chronic active	115/70		8.5	62.2	83	206	139	
			April 25, 1932	Chronic active	115/15	67	79.8	91*	17	30	20	
119-975	F	38	November 21, 1932	Chronic active	110/100	18	24.2	29.1*	51	89	60	
119-722	M	15	October 8, 1931	Chronic active	115/80	20	28.8	11.6	19	28	19	
119-916	F	21	November 22, 1932	Chronic active	160/110	50	17.6	33.1	15	77	52	
150-097	M	32	November 29, 1932	Chronic active	105/70	65	21.1	18.8*	35	62	12	
			December 9, 1932	Chronic active		70	11.2	50.0*	93	95	64	

TABLE III—(Continued)

Hospital number	Sex	Age years	Date	Stage of disease	Blood pressure mm Hg	Phenol sulphon phthalein per cent in 2 hours	Blood urea nitrogen mgm per cent	Urea clearance cc. per minute	Per cent of normal	Creatinine clearance cc. per minute	Per cent of normal	Date of death
151-107	F	35	February 10 1933	Chronic active	120/70	58	16.0	44.0*	82	79	53	
118-347	M	54	October 2 1931	Chronic active	130/80	60	7.5	67.0	89	88	60	
			November 2 1931	Chronic active	140/100	50	11.3	34.9	47	47	32	
142-123	M	51	September 28, 1931	Chronic active	155/85	60	19.0	24.4	33	70	47	
144-018	F	28	December 8, 1931	Chronic active	165/90	65	14.9	24.6*	46	53	36	
142-730	M	34	October 2, 1931	Chronic active	185/110	40	14.0	18.2*	34	47	32	
			October 10 1932	Chronic active	205/135	10	30.3	13.8*	26	29	20	
			January 4 1933	Terminal	190/130	10	28.6	14.9	20	20	14	March 1, 1933
86-642	F	31	October 28 1932	Chronic active	110/80	25	24.0	11.9	16	28	19	
			January 25 1933	Chronic active			24.7	9.3	12	26	18	
145-429	M	43	March 1 1932	Latent	135/80	70	14.0	101.0	135	149	101	
0-343-184	F	59	April 6, 1933	Latent	160/100		8.9	67.0	81	126	85	
134-772	M	52	July 20 1931	Terminal	160/110		12.4	21.5*	40	46	31	November 28 1932
89-697	M	22	February 4, 1932	Terminal	180/110	5	11.0	3.9*	7	6	5	May 6, 1932
145-811	M	49	March 28 1932	Terminal	140/70	5	188.0	0.6*	1	0.5	0.34	April 1, 1932
117-565	F	20	October 19 1931	Terminal	150/110	10	52.4	3.8*	7	8.0	5.0	February 21, 1933
151-508	F	27	March 3 1933	Terminal	260/150	10	70.0	5.5*	10	12.0	8	March 4, 1933
147-812	F	49	July 26 1932	Terminal	110/60	5	119.0	0.9*	2	0.4	0.3	July 28 1932

* Standard clearances

TABLE III
Creatinine and urea clearances in patients with hemorrhagic Bright's disease

Hospital number	Sex	Age	Date	Stage of disease	Blood pressure mm Hg	Phenol-sulphon phtalein per cent in 2 hours	Blood urea nitrogen mgm per cent	Urea clearance cc. per minute	Per cent of normal	Creatinine clearance cc. per minute	Per cent of normal	Date of death
116-625	F	11	May 17, 1932	Acute	110/70	80	16.5	32.8	41	55	37	
116-212	M	33	January 28, 1932	Acute	110/100		33.6	51.0	68	94	64	
136-555	M	20	January 26, 1933	Acute	135/80	55	10.0	32.8*	61	83	56	
118-341	M	55	October 26, 1932	Acute	110/80	30	25.3	33.3	41	80	51	
150-922	F	27	February 23, 1933	Acute	115/80		9.8	92.5	123	166	112	
139-831	M	12	June 25, 1931	Acute	190/120	20	34.9	12.7*	21	39	26	
			August 27, 1931	Latent	110/90	70	14.0	21.9*	41	69	47	
			December 22, 1932	Latent	120/80		18.0	36.9	49	88	59	
			January 5, 1933	Latent			9.7	34.2	16	73	19	
			January 19, 1933	Latent			21.7	43.2	58	95	64	
			January 23, 1933	Latent			34.1	22.3*	41	66	15	
			January 30, 1933	Latent			9.2	27.8	37	73	49	
150-571	M	38	January 1, 1933	Chronic active	180/100	68	18.2	21.1	29	41	28	
116-216	M	52	April 26, 1932	Chronic active	130/80	30	26.6	16.6*	31	86	58	
107-624	M	15	January 12, 1932	Chronic active	115/70		8.5	62.2	83	206	139	
			April 25, 1932	Chronic active	115/45	67	79.8	9.1*	17	30	20	
119-975	F	38	November 21, 1932	Chronic active	140/100	48	24.2	29.1*	54	89	60	
139-722	M	15	October 8, 1931	Chronic active	115/80	20	28.8	11.6	19	28	19	
119-916	F	24	November 22, 1932	Chronic active	160/110	50	17.6	33.1	45	77	52	
150-097	M	32	November 29, 1932	Chronic active	105/70	65	21.1	18.8*	35	62	12	
			December 9, 1932	Chronic active		70	14.2	50.0*	93	95	64	

TABLE III—(Continued)

Hospital number	Sex	Age years	Date	Stage of disease	Blood pressure mm Hg	Phenol sulphon phthalein per cent in 2 hours	Blood urea nitrogen mgm per cent	Urea clearance cc per minute	Per cent of normal	Creatinine clearance cc per minute	Per cent of normal	Date of death
151-107	F	35	February 10 1933	Chronic active	120/70	58	16.0	44.0*	82	79	53	
118-347	M	54	October 2, 1931	Chronic active	130/80	60	7.5	67.0	89	88	60	
			November 2 1931	Chronic active	140/100	50	11.3	34.9	47	47	32	
142-123	M	51	September 28 1931	Chronic active	155/85	60	19.0	24.4	33	70	47	
144-018	F	28	December 8 1931	Chronic active	165/90	65	14.9	24.6*	46	53	36	
142-730	M	34	October 2, 1931	Chronic active	185/110	40	14.0	18.2*	34	47	32	
			October 10 1932	Chronic active	205/135	10	30.3	13.8*	26	29	20	
			January 4 1933	Terminal	190/130	10	28.6	14.9	20	20	14	March 1 1933
86-642	F	31	October 28 1932	Chronic active	110/80	25	24.0	11.9	16	28	19	
			January 25 1933	Chronic active			24.7	9.3	12	26	18	
145-429	M	43	March 1 1932	Latent	135/80	70	14.0	101.0	135	149	101	
0-343-184	F	59	April 6 1933	Latent	160/100		8.9	67.0	81	126	85	
134-772	M	52	July 20, 1931	Terminal	160/110		12.4	21.5*	40	46	31	November 28, 1932
89-697	M	22	February 4 1932	Terminal	180/110	5	111.0	3.9*	7	6	5	May 6, 1932
145-811	M	49	March 28 1932	Terminal	140/70	5	188.0	0.6*	1	0.5	0.34	April 1 1932
117-565	F	20	October 19 1931	Terminal	150/110	10	52.4	3.8*	7	80	50	February 21, 1933
151-508	F	27	March 3 1933	Terminal	260/150	10	70.0	5.5*	10	120	8	March 4, 1933
147-812	F	49	July 26 1932	Terminal	110/60	5	119.0	0.9*	2	0.4	0.3	July 28 1932

* Standard clearances

TABLE IV
Creatinine and urea clearances in patients with arteriosclerotic Bright's disease

Hospital number	Sex	Age, years	Date	Blood pressure, mm Hg	Phenol-sulphon phthalein, per cent in 2 hours	Blood urea nitrogen, mgm. per cent	Urea clearance, cc. per minute	Per cent of normal	Creatinine clearance, cc. per minute	Per cent of normal	Date of death
0-318-091	F	41	April 6, 1933	220/110		13.5	46.8	62	116	78	
105-700	M	41	January 19, 1933	260/160	30	13.2	41.7*	77	111	75	
126-997	M	51	October 6, 1931	150/110	50	11.2	31.8	46	98	66	June 28, 1932
115-997	M	29	April 7, 1932	200/130	52	16.9	31.8*	59	98	66	
			July 29, 1932	230/170	25	31.9	26.8*	50	47	32	August 27, 1932
119-731	F	60	November 23, 1932	110/80		15.4	44.8*	83	91	62	
0-339-611	M	62	January 17, 1933	200/100	55	20.0	37.8*	70	81	55	
150-795	F	28	January 18, 1933	198/130	56	22.5	72.6	97	78	53	
113-350	F	38	May 17, 1932	210/150	10	29.1	34.6	16	73	19	
87-720	F	30	November 9, 1932	300/165	55	15.2	29.1	39	71	18	
135-951	F	44	January 12, 1933	275/151	30	20.1	34.5*	64	65	44	
149-462	F	55	November 8, 1932	235/155	40	16.5	13.7*	25	59	40	
150-053	F	56	November 29, 1932	200/100	70	19.6	16.4*	30	59	40	
113-018	M	43	June 28, 1932	150/90		23.1	15.8*	29	55	37	
138-277	F	49	January 2, 1931	170/95	10	20.5	40.1*	75	55	37	
			October 14, 1932	180/100	10	36.4	3.2*	6	7	5	
			January 10, 1933	165/90	10	47.0	5.1*	9	12	8	
121-988	M	41	July 16, 1932	190/125	55	20.3	10.3*	19	52	35	

TABLE IV (Continued)

Hospital number	Sex	Age years	Date	Blood pressure mm Hg	Phenol sulphon phthalein per cent in 2 hours	Blood urea nitrogen mgm per cent	Urea clearance cc per minute	Per cent of normal	Creatinine clearance cc per minute	Per cent of normal	Date of death
151-530	M	52	March 6 1933	220/30	50	22.8	23.4*	43	45	30	November 9 1932
141-842	M	56	June 28 1932	260/140	25	29.8	19.2*	36	45	30	
125-983	M	52	September 2 1932	170/120	40	17.4	28.1*	52	40	27	
144-079	F	38	October 11 1932	225/145	33	35.0	11.2*	21	35	24	
150-478	M	39	January 9 1933	220/135	25	40.6	15.4*	29	34	23	
142-341	F	38	March 14 1933	245/150	15	53.5	8.1*	15	13	9	September 27 1931
142-324	M	55	September 2 1931	170/116	5	18.0	25.6	34	32	22	
146-857	M	30	September 25, 1931	240/140	5	74.1	29.2*	54	32	22	
148-705	F	48	July 6 1932	220/160	10	109.0	0.7*	1	11	7	
149-634	M	44	August 23 1932	245/150	18	49.7	18.6	25	27	18	
149-237	M	58	August 31 1932	200/160	5	105.7	16.5	22	21	14	August 2 1932
125-448	F	43	November 8 1932	260/130	10	57.4	12.9	17	18	12	December 30 1932
150-929	F	60	October 5 1932	150/90	10	76.3	5.2*	10	15	10	November 20, 1932
134-806	F	32	September 24 1932	160/110	5	46.9	17.5*	32	11	7	December 11 1932
144-535	F	45	October 13 1932	170/80	5	156.0	5.0*	9	7	5	January 23 1933
146-471	M	56	January 22 1933	230/140	3	61.5	3.8*	7	7	5	August 19 1931
91-504	F	43	July 22, 1931	230/110	3	131.5	4.1*	8	4	3	January 8, 1932
147-168	F	40	January 7 1932	160/100	5	232.0	1.7*	0.6	3	2	January 19 1932
141-832	M	47	May 19 1932	250/170	3	118.0	0.3*	7.0	3	2	May 19 1932
			June 9, 1932	230/110	5	88.9	3.6*	3.0	2	1.4	June 18 1932
			July 17 1932	235/130		163.0	1.7*	0.4	0.6	0.4	June 29 1932
			July 31 1931				0.2*				August 5 1931

* Standard clearances

TABLE V
Creatinine and urea clearances in patients with degenerative Bright's disease and certain other diseases

Hospital number	Sex	Age	Date	Diagnosis	Blood pressure	Phenol sulphophthalein	Blood urea nitrogen	Urea clearance	Per cent of normal	Creatinine clearance	Per cent of normal	Date of death
		years			mm Hg	per cent in 3 hours	mgm per cent	cc per minute		cc per minute		
150-775	F	23	January 12, 1932	Degenerative Bright's disease and eclampsia	155/100	55	12.5	23.7*	41	56	38	
			March 20, 1933	Degenerative Bright's disease			13.7	31.3	12	77	52	
151-532	M	41	March 6, 1933	Degenerative Bright's disease	150/95	12	26.1	29.6	39	41	28	
151-057	F	21	February 1, 1933	Degenerative Bright's disease and eclampsia	165/120		60.1	17.5*	32	21	11	
			April 3, 1933	Degenerative Bright's disease	158/100	50	13.1	25.2*	17	61	41	
110-975	F	38	December 29, 1932	Diabetes and arteriosclerosis	150/90	35	27.3	33.0*	61	131	89	
110-793	M	53	January 21, 1933	Diabetes and arteriosclerosis	150/100	26	32.2	18.1	21	32	22	
0-320-284	M	13	January 14, 1933	Diabetes and arteriosclerosis	240/110		19.7	19.6*	36	51	31	
111-380	F	19	January 27, 1932	Diabetes and arteriosclerosis	230/130	40	9.1	12.0*	78	65	11	
136-525	M	28	October 15, 1932	Diabetes insipidus	130/85		11.0	38.5	51	101	70	August
				Diabetes insipidus			15.0	13.5	58	79	53	27, 1932

TABLE V (Continued)

Hospital number	Sex	Age	Date	Diagnosis	Blood pressure	Phenol sulphthalein	Blood urea nitrogen	Urea clearance	Per cent of normal	Creatinine clearance	Per cent of normal	Date of death
		years			mm Hg	per cent in 3 hours	mgm per cent	cc per minute		cc per minute		
133-845	M	23	August 22 1932	Essential Hypertension	160/110	80	8.1	103.0	137	119	80	November 30, 1932
149-816	F	67	November 28, 1932	Carcinoma of stomach and arteriosclerosis	125/70		56.4	11.8*	22	44	30	
149-535	M	64	December 5, 1932	Carcinoma of bronchus	110/70		15.0	21.1*	39	74	50	
122-258	F	66	June 10 1932	Carcinoma of stomach	170/90		6.5	14.5*	27	28	19	June 21, 1932
146-484	M	75	May 6 1932	Carcinoma of prostate	110/55	10	16.9	28.7*	53	81	55	May 9 1932
149-077	F	52	November 25 1932	Carcinoma of pancreas and jaundice	90/60		7.0	37.0*	69	20	14	November 30, 1932
146-070	M	50	April 7 1932	Pneumonia	110/65		19.2	63.5	85	88	59	September 14 1931
142-587	M	26	September 12 1931	Pneumonia	125/75		41.4	30.0*	56	145	98	September 27, 1931
142-828	M	36	September 25 1931	Pneumonia	130/70		15.0	62.0*	115	155	105	March 18, 1932
145-644	M	27	March 11 1932	Pneumonia	95/60		19.5	76.0*	141	231	156	January 13 1933
150-738	M	51	January 12 1933	Pneumonia	110/80		65.0	8.3*	15	38	26	November 18 1931
143-756	M	36	November 16, 1931	Pneumonia			183.0	9.45	13	23	16	December 18, 1931
144-053	M	71	December 8, 1931	Pyelonephritis	125/80	5	137.0	3.5	5	5	3	August 24 1932
147-549	F	34	July 19, 1932	Pyelonephritis	108/78	20	73.5	7.0*	13	9	6	March 18, 1932
85-756	F	70	March 13 1933	Pyelonephritis	130/76	Trace	175.0	0.8*	1.5	2	1.4	

TABLE V (Continued)

Hospital number	Sex	Age, years	Date	Diagnosis	Blood pressure, mm Hg	Phenol sulphation phthalate, per cent in 2 hours	Blood urea nitrogen, mgm per cent	Urea clearance, cc per minute	Per cent of normal	Creatinine clearance, cc per minute	Per cent of normal	Date of death
111-710	M	50	January 18, 1932	Bismuth poisoning and pyelonephritis	130/75	Trace	217.0	1.4*	3	2	1.1	January 28, 1932
111-816	F	39	November 30, 1931	Arteriosclerosis	180/140	80	7.8	68.0	91	147	99.0	
125-080	F	71	May 13, 1932	Arteriosclerosis	130/85	43	11.1	31.6*	59	157	106.0	
151-612	M	76	March 21, 1933	Hypertrophied prostate	140/80	85	17.6	37.8	50	68	16	
			April 14, 1933	Hypertrophied prostate			16.5	42.6	57	68	16	
151-583	M	65	March 21, 1933	Hypertrophied prostate	120/90	40	57.2	12.1	16	21	14	
			April 5, 1933	Hypertrophied prostate			32.3	27.3	36	41	30	
116-177	M	59	April 11, 1932	Pernicious anemia	115/55		10.8	44.8*	83	98	66	
150-871	F	38	February 14, 1933	Cirrhosis of liver and jaundice	110/70		14.5	39.4*	73	57	39	
111-512	M	50	March 15, 1933	Subacute endocarditis	160/90	15	30.6	24.3	32	24	16	
111-891	F	19	August 26, 1931	Streptococcal septicemia and acute interstitial nephritis	105/60	Trace	88.7	2.5*	5	3	2	September 8, 1931

* Standard clearances

standard urea clearance, however, may have a greater numerical value than the creatinine clearance determined at the same time, since the concentration ratio is multiplied by the square root of urine volume in the former and by the volume in the latter

A direct comparison of the two clearance tests is shown in Figure 2 in which the results of each test are plotted as percentage of average normal

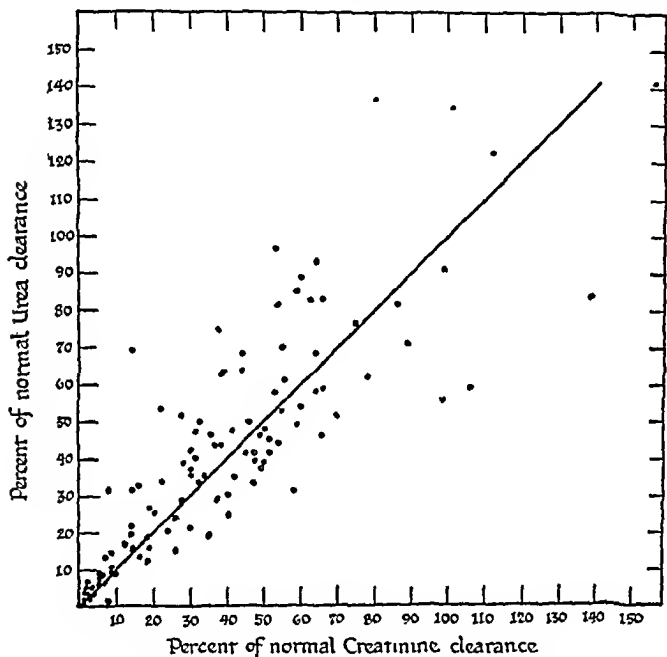


FIG 2 COMPARISON OF CREATININE AND UREA CLEARANCE TESTS IN PATIENTS WITH BRIGHT'S DISEASE AND CERTAIN OTHER CONDITIONS

Points above the line indicate instances in which the urea clearance showed less reduction than creatinine, those below instances in which the percentage reduction in urea clearance was greater. In patients with creatinine clearances below 10 per cent of average normal there is not much difference in the degree of reduction of the two tests. In 82 creatinine clearances between 10 and 80 per cent of average normal, the urea clearance was reduced to a greater degree in 32, while in 50 it was not reduced to the same extent. These differences are of questionable significance when the

TABLE V (Continued)

Hospital number	Sex	Age, years	Date	Diagnosis	Blood pressure mm Hg	Phenol sulphon phthalein per cent in 2 hours	Blood urea nitro- gen mgm per cent	Urea clear- ance cc per minute	Per cent of normal	Creati- nine clear- ance cc per minute	Per cent of normal	Date of death
111-710	M	50	January 18, 1932	Bismuth poisoning and pyelonephritis	130/75	Trace	217.0	1.4*	3	2	11	January 28, 1932
113-816	F	39	November 30, 1931	Arteriosclerosis	180/110	80	7.8	68.0	91	147	99.0	
125-080	F	34	May 13, 1932	Arteriosclerosis	130/85	43	11.1	31.6*	59	157	106.0	
151-612	M	76	March 21, 1933	Hypertrophied prostate	110/80	85	17.6	37.8	50	68	16	
			April 14, 1933	Hypertrophied prostate			16.5	42.6	57	68	16	
151-583	M	65	March 21, 1933	Hypertrophied prostate	120/90	40	57.2	12.1	16	21	11	
			April 5, 1933	Hypertrophied prostate			32.3	27.3	36	41	30	
116-177	M	59	April 14, 1932	Periculous anemia	115/55		10.8	41.8*	83	98	66	
150-874	F	38	February 14, 1933	Cirrhosis of liver and jaundice	110/70		14.5	39.4*	73	57	39	
111-512	M	50	March 15, 1933	Subacute endo- carditis	160/90	15	30.6	21.3	32	24	16	
111-891	F	19	August 26, 1931	Streptococcal septi- cemia and acute interstitial nephri- tis	105/60	Trace	88.7	2.5*	5	3	2	September 8, 1931

* Standard clearances

- 9 Shannon J A, Jolliffe, N, and Smith, H W, *Am J Physiol* 1932, *cu* 534 The Excretion of Urine in the Dog VI The Filtration and Secretion of Exogenous Creatinine
- 10 MacKay, E M *J Clin Invest*, 1929, *vi*, 505 Studies of Urea Excretion V The Diurnal Variation of Urea Excretion in Normal Individuals and Patients with Bright's Disease
- 11 Van Slyke, D D, *J Biol Chem*, 1927, *lxxiii*, 695 Determination of Urea by Gasometric Measurement of the Carbon Dioxide Formed by the Action of Urease
- 12 Van Slyke, D D, and Cullen, G E, *J Biol Chem*, 1916, *xxiv* 117 The Determination of Urea by the Urease Method
- 13 Rehberg, P B, *Zentralbl f inn Med*, 1929, *i*, 367 Über die Bestimmung der Menge des Glomerulusfiltrats mittels Kreatinin als Nierenfunktionsprüfung, nebst einigen Bemerkungen über die Theorien der Harnbereitung
- 14 Behre J A, and Benedict S R, *J Biol Chem* 1922 *lxi* 11 Studies in Creatine and Creatinine Metabolism IV On the Question of the Occurrence of Creatinine and Creatine in Blood
- 15 Gaebler, O H, *J Biol Chem* 1930 *lxxxix*, 451 Further Studies of Blood Creatinine
- 16 Gaebler O H, and Keltch, A K *J Biol Chem* 1928, *lxxvi*, 337 On the Nature of Blood Creatinine
- 17 McIntosh, J F, Moller, E, and Van Slyke, D D, *J Clin Invest*, 1928, *vi*, 467 Studies of Urea Excretion III The Influence of Body Size on Urea Output
- 18 Taylor, F B, Drury D R, and Addis, T, *Am J Physiol*, 1923 *lxv*, 55 The Regulation of Renal Activity VIII The Relation Between the Rate of Urea Excretion and the Size of the Kidneys
- 19 Holten, C and Rehberg P B *Acta med Scandinav*, 1931 *lxxiv* 479 Studies on the Pathological Function of the Kidneys in Renal Disease, Especially Bright's Disease I
- 20 Addis T J A M A, 1925, *lxxxv*, 163 A Clinical Classification of Bright's Disease
- 21 Van Slyke D D, McIntosh, J F Möller E Hannon, R R, and Johnston, C, *J Clin Invest* 1930, *viii*, 357 Studies of Urea Excretion VI Comparison of the Blood Urea Clearance with Certain Other Measures of Renal Function

wide range of normal variation in both tests is remembered. The most that can be said in favor of the creatinine test is that it may indicate a greater decrease in function more frequently than the urea clearance, but not with enough regularity to make up for the greater technical difficulty of the test. Nor have we been able to find any group of patients or any pathological condition in which the results of one test are consistently different from those of the other.

Since the creatinine test is more laborious and expensive, involving the ingestion of creatinine and the analysis of two blood samples, we do not believe it has any advantage in the routine estimation of the degree of impairment of kidney function in the clinic. If it can be satisfactorily shown that the creatinine clearance does approximate the volume of glomerular filtrate, then a comparison of the two tests, run simultaneously, will permit a much more intimate analysis of the parts played by variations in the volume of filtrate and degree of back diffusion in health and disease (19).

CONCLUSIONS

The creatinine and urea clearance tests have been compared in normal persons and in patients with Bright's disease. The mean creatinine clearance in 130 observations of 59 normal subjects was 148 cc per minute. The variability of the two tests from the mean normal was approximately the same in our hands.

In patients with Bright's disease the creatinine and urea clearance tests are generally equally reduced in relation to the average normal. We were unable to demonstrate any practical advantage in the creatinine test to compensate for its greater technical difficulty.

BIBLIOGRAPHY

- 1 Möller, E, McIntosh, J F, and Van Slyke, D D, J Clin Invest, 1928, vi, 427. Studies of Urea Excretion. II Relationship Between Urine Volume and the Rate of Urea Excretion by Normal Adults.
- 2 Rehberg, P B, Biochem J, 1926, xx, 447. Studies on Kidney Function. I The Rate of Filtration and Reabsorption in the Human Kidney.
- 3 Wyschegorodzewa, V D, Ztschr f d ges exper Med, 1931, lxxvi, 72. Zur Bestimmung der Nierenfunktion auf Grund der modernen Filtrations-Reabsorptionstheorie der Harnabsonderung.
- 4 Bergwall, A, Klin Wchnschr, 1932, vi, 554. Glomerulusfiltratmengenbestimmungen bei Nierenkranken.
- 5 Hemingway, A, J Physiol, 1933, lxxvii, 13P. The Rate of Glomerular Filtration in the Perfused Kidney.
- 6 Ellis, L B, and Weiss, S, J A M A, 1933, c, 875. Renal Function in Arterial Hypertension.
- 7 Cope, C L, Quart J Med, 1931, xxiv, 567. The Excretion of Creatinine by the Human Kidney in Health and in Nephritis.
- 8 Ekenroth, G, Acta med Scandinau, 1931, Supplement xxxvi. On the Principles of Renal Function.

EXPERIMENTS ON THE RELATION OF CREATININE AND UREA CLEARANCE TESTS OF KIDNEY FUNCTION AND THE NUMBER OF GLOMERULI IN THE HUMAN KIDNEY OBTAINED AT AUTOPSY

BY J M HAYMAN JR AND S M JOHNSTON

*(From the H K Cushing Laboratory of Experimental Medicine in the Department
of Medicine of Western Reserve University and the Medical
Service of Lakeside Hospital Cleveland)*

(Received for publication May 1 1933)

Correlation of the results of tests of renal function with the pathological changes in the kidney has proved extremely difficult To cite a simple example in the marked proteinuria of degenerative Bright's disease, lesions of the glomeruli may be either slight or undiscoverable, while the tubule cells are strikingly abnormal And yet, by far the greatest part of the protein is believed to have been excreted through the glomeruli Similar difficulties have led many students to the conclusion that alterations of kidney function are independent of the nature of the anatomical changes in the kidney Fortunately for the clinician, the results of kidney function tests can be expressed in relation to the normal, without regard to the nature or the anatomical site of the condition which has produced the abnormality The average amount of urea excreted per minute by a normal individual with high urine volumes is equal to the urea contained in 75 cc of blood (1) If it is only equivalent to that contained in 20 cc of blood kidney function may be said to be reduced without any intimation whether this reduction is brought about by diminished blood flow decrease in the amount of renal tissue increased reabsorption of urea by the tubules or diminished secretion But an attempt to discover the mechanism of such reductions in function is irresistible to one concerned with the study of kidney disease

Van Slyke and his collaborators (2), from a comparison of "terminal" urea clearance values and autopsy findings suggested that in hemorrhagic and degenerative Bright's disease one may interpret the blood urea clearance as a measure of the proportion of glomerular tissue still functioning while in arteriolar sclerosis it appeared proportional to the decrease in renal blood flow rather than to the glomerular destruction Addis (3) used the ratio (urea in 1 hour's urine/blood urea concentration) (which under his conditions can be converted into maximum urea clearance by multiplying by 1.67), as a measure of the extent of the renal lesion—"the amount

little difference in the proportion of uninjected glomeruli. Any kidney in which a visible infarct was produced was discarded, or cut out and weighed and allowance made for it. It is assumed that the glomeruli are uniformly distributed throughout the suspension. Thorough mixing before sampling is essential. The variation in the counts of different aliquots is chiefly due to insufficient mixing. From 3 to 20 aliquots were counted, the standard deviation calculated and the estimate shown to be significant according to Fisher's (8) method of calculation¹. It is further assumed that the numbers of glomeruli in the two kidneys of the same individual are equal within about 10 per cent. This was shown for the rabbit by Hayman and Starr (9), for the human by Moore (10).

Estimations of the number of glomeruli in the normal human kidney vary from 560 000 to 5,700,000 by different methods. By injection of iron salts and acid digestion Vimtrup (11) found 833,992 to 1,233,360 and Moore 800,000 to 1,000,000 with occasional values as low as 600 000 and as high as 1,200,000. Our series includes 12 kidneys from persons without histological evidence of significant abnormalities in the glomeruli or blood vessels. The counts ranged from 800,000 to 1 530,000. The mean was 1,156 000 and the standard deviation 191 266. This means that counts differing by more than twice the standard deviation, or below 773,000, probably indicate a significant decrease in the number of glomeruli, while those differing by more than three times the standard deviation or below 582 000 certainly do.

Table I shows the comparison of creatinine and urea clearance tests with the estimated number of glomeruli in one kidney of 34 persons. We have followed Addis's classification of Bright's disease. In arteriosclerotic Bright's disease, decrease in clearances was associated with progressive reduction in the number of injected glomeruli. There were only 2 patients with active hemorrhagic Bright's disease and in one of these the estimate of the number of glomeruli is not significantly low. The other showed about the same reduction in clearance tests and glomeruli as the sclerotic group. Since in both chronic hemorrhagic Bright's disease and arterio-sclerotic Bright's disease the patency of the renal vessels is reduced to

¹ An example of the method of calculation. Hospital number 149-512. Weight of kidney before perfusion 163 grams after perfusion 254 grams. Weight of pieces taken for histological section 2 grams. After digestion diluted to 16 liters. Counts on 2 cc aliquots 153 155 147 189 118 148, 154, 96 117, 111. Mean 138.8. Standard deviation 27. From these data, *t* calculated by the method of Fisher is 15.85 and the mean therefore significant. Estimate of total from mean 1,110 400. Correction for 2 grams taken for histological sections 8 812. Corrected estimate of count 1 119 212. In a count of 1 075 glomeruli from 3 blocks in sections so spaced that no glomerulus was counted twice, 1 052 contained injection mass. 23 apparently patent glomeruli did not. Correction for uninjected patent glomeruli 24 465. Final estimate of patent glomeruli 1 143 400.

of renal tissue which has been rendered functionless by the disease process." He did not attempt to distinguish between actual decrease in mass of tissue present and decreased accomplishment of a normal kidney mass. Thus should the ratio be reduced, let us say half normal, by removal of one kidney or by poisoning both kidneys even by a poison from which recovery could take place the two results would be considered the same—in each half the renal tissue had ceased to work.

Decrease in the creatinine clearance test has usually been ascribed to decrease in the volume of glomerular filtrate (4). Such a reduction could be due to decrease in blood flow, blood pressure or filtering surface. On the other hand, if the tubule cells were so damaged that they were unable to resist back diffusion of creatinine filtered through the glomeruli, or to secrete the constant fraction of the amount filtered as Shannon, Jolliffe and Smith believe (5), there might be a reduction in creatinine clearance without any decrease in the amount of glomerular filtrate. In such circumstance, it seems difficult to relate the decrease directly to reduction in functioning mass of renal tissue.

In the hope of furthering a physiological interpretation of the creatinine and urea clearance tests, we have estimated the number of glomeruli in one kidney of a number of patients whose clearances had been measured from one day to several months before death. All of the patients had systolic blood pressures above 100 mm Hg and were without evidence of congestive heart failure at the time the clearance tests were made. When there was any doubt of the ability to empty the bladder, the patient was catheterized at the beginning and end of the period of urine collection. The tests were made in the manner described in the preceding paper (6).

If there was any gross difference in the pathological condition of the two kidneys, the experiment was discarded. The number of glomeruli was estimated by Kunkel's (7) modification of Vimtrup's method. The kidney was perfused at 140 mm Hg pressure with a mixture of equal parts of 2.5 per cent potassium ferrocyanide and ferric ammonium citrate, after washing out the blood with 0.9 per cent NaCl. It was then cut in pieces, digested in 50 per cent HCl for 10 to 36 hours, washed in tap water, and allowed to stand in distilled water for 24 to 48 hours. When sufficiently digested, it was diluted to a suitable volume and thoroughly mixed. The number of glomeruli in 2 cc aliquots was then counted on a ruled watch glass under a binocular microscope. In the majority of instances, the completeness of injection has been checked by histological sections, and the estimated number of glomeruli corrected for the proportion of apparently patent but uninjected glomeruli.

The use of such an estimate of the number of glomeruli involves several assumptions. It is assumed that the proportion of uninjected glomeruli is nearly the same throughout the kidney. Counts of injected and uninjected glomeruli in blocks taken from different parts of the kidney show

little difference in the proportion of uninjected glomeruli. Any kidney in which a visible infarct was produced was discarded, or cut out and weighed and allowance made for it. It is assumed that the glomeruli are uniformly distributed throughout the suspension. Thorough mixing before sampling is essential. The variation in the counts of different aliquots is chiefly due to insufficient mixing. From 3 to 20 aliquots were counted the standard deviation calculated and the estimate shown to be significant according to Fisher's (8) method of calculation¹. It is further assumed that the numbers of glomeruli in the two kidneys of the same individual are equal within about 10 per cent. This was shown for the rabbit by Hayman and Starr (9), for the human by Moore (10).

Estimations of the number of glomeruli in the normal human kidney vary from 560,000 to 5,700,000 by different methods. By injection of iron salts and acid digestion Vimtrup (11) found 833,992 to 1,233,360, and Moore 800,000 to 1,000,000 with occasional values as low as 600,000 and as high as 1,200,000. Our series includes 12 kidneys from persons without histological evidence of significant abnormalities in the glomeruli or blood vessels. The counts ranged from 800,000 to 1,530,000. The mean was 1,156,000 and the standard deviation 191,266. This means that counts differing by more than twice the standard deviation, or below 773,000, probably indicate a significant decrease in the number of glomeruli, while those differing by more than three times the standard deviation, or below 582,000 certainly do.

Table I shows the comparison of creatinine and urea clearance tests with the estimated number of glomeruli in one kidney of 34 persons. We have followed Addis's classification of Bright's disease. In arteriosclerotic Bright's disease, decrease in clearances was associated with progressive reduction in the number of injected glomeruli. There were only 2 patients with active hemorrhagic Bright's disease and in one of these the estimate of the number of glomeruli is not significantly low. The other showed about the same reduction in clearance tests and glomeruli as the sclerotic group. Since in both chronic hemorrhagic Bright's disease and arteriosclerotic Bright's disease the patency of the renal vessels is reduced to

¹ An example of the method of calculation. Hospital number 149-512. Weight of kidney before perfusion 163 grams, after perfusion 254 grams. Weight of pieces taken for histological section, 2 grams. After digestion diluted to 16 liters. Counts on 2 cc aliquots 153 155, 147 100, 148, 154 96 117, 111. Mean 138.8. Standard deviation 22. From calculated by the method of Fisher, is 15.85 and the mean therefore Estimate of total from mean 1,110,400. Correction for 2 grams histological sections 8812. Corrected estimate of count 1,119,512. of 1,075 glomeruli from 3 blocks in sections so counted twice, 1,052 contained injection mass, 23 apparently patent, did not. Correction for uninjected patent glomeruli 24,463. Final patent glomeruli 1,143,400.

TABLE I

Relation of creatinine and urea clearance tests and the number of glomeruli in one kidney

Case number	Hospital number	Sex	Age	Diagnosis	Creatinine clearance	Urea clearance	Glomeruli in one kidney
			years		cc per minute	cc per minute	
1	143-651	F	34	Miliary tuberculosis	145	40.6*	1,260,000
2	149-586	F	23	Miliary tuberculosis	201	112.0	1,230,000
3	145-915	F	15	Tuberculous meningitis	129	66.7*	1,061,000
4	136-525	M	28	Diabetes insipidus	104	39.0	1,288,000*
5	147-691	M	36	Miliary tuberculosis	108	36.0*	800,000*
6	146-212	M	33	Tuberculous meningitis	94	51.0	896,000
7	145-644	M	27	Pneumonia	231	76.0*	1,250,000
8	142-828	M	36	Pneumonia	151	62.0*	1,037,000
9	142-587	M	26	Pneumonia	145	26.0*	1,336,000
10	150-738	M	51	Pneumonia	38	8.3*	1,530,000
11	143-756	M	36	Pneumonia	23	9.5	1,250,000
12	146-484	M	75	Carcinoma of prostate and arteriosclerotic Bright's disease	81	28.7*	1,056,000
13	149-816	F	67	Carcinoma of stomach and arteriosclerotic Bright's disease	44	11.8*	402,000
14	144-079	F	38	Arteriosclerotic Bright's disease	35	11.2*	490,000
15	145-997	M	29	Arteriosclerotic Bright's disease	47	27.0*	390,000
16	142-324	M	53	Arteriosclerotic Bright's disease	32	29.0*	174,000
17	149-143	M	54	Arteriosclerotic Bright's disease	30		341,000
18	143-018	F	25	Arteriosclerotic Bright's disease	27	12.3*	413,000
19	149-634	M	44	Arteriosclerotic Bright's disease	18	13.0	422,000
20	149-237	M	58	Arteriosclerotic Bright's disease	15	5.0	240,000
21	134-806	F	32	Arteriosclerotic Bright's disease	7	4.0*	359,000
22	91-504	F	43	Arteriosclerotic Bright's disease	3	3.6*	275,000
23	146-741	M	56	Arteriosclerotic Bright's disease	3	0.3*	168,000
24	147-168	F	40	Arteriosclerotic Bright's disease	2	1.7*	192,000
25	145-306	F	43	Arteriosclerotic Bright's disease	1		156,000
26	141-832	M	47	Arteriosclerotic Bright's disease	0.6	0.2*	195,000
27	149-512	M	21	Focal hemorrhagic Bright's disease	126	39.0*	1,143,000
28	141-891	F	19	Acute hemorrhagic Bright's disease	3	2.5*	819,000
29	142-730	M	36	Active hemorrhagic Bright's disease	29	13.8*	746,000
30	151-508	F	27	Active hemorrhagic Bright's disease	12	5.5*	170,000
31	144-740	M	50	Pyelonephritis with multiple abscesses and arteriosclerotic Bright's disease	2	1.4*	393,000
32	144-053	M	71	Hypertrophied prostate and pyelonephritis with multiple abscesses	5	3.5	490,000
33	147-594	F	34	Pyelonephritis and B. coli septicemia	9	6.9*	1,330,000
34	149-077	F	52	Carcinoma of pancreas and bile nephrosis	3		645,000

* Standard clearance

about the same degree (12) and since not only is the final clinical picture often very similar but even the pathological distinction at times difficult, it seems that in both groups the reduction in clearances is probably due to reduction in number of glomeruli.

In patients with acute nephritis who show decreased clearance values with return toward normal during convalescence it seems reasonable to suppose that certain glomeruli have been rendered temporarily functionless by inflammation, but that the process has not gone on to destruction so that recovery can take place. The one patient in the series with acute hemorrhagic nephritis had a very low clearance, but a normal number of glomeruli. The injection method of estimating glomeruli does not, of course, distinguish between normal and abnormal glomeruli since any whose capillaries are sufficiently patent to be injected at the pressure used must be counted. Sections of the other kidney from this patient showed degenerative or inflammatory changes in nearly every glomerulus.

Møller, McIntosh and Van Slyke attribute decrease in the volume of blood cleared of urea per minute in pathological conditions to one of two causes: either a decrease in the volume of blood passing through the kidneys, or a decrease in the proportion of its urea removed during the passage. In cardiac decompensation, glomerular nephritis and arteriolar sclerosis diminished flow seems certain. Whether a decrease in the proportion of urea removed from the blood also occurs they felt there was at present no basis to surmise. Holten and Rehberg on the other hand believe from comparison of creatinine and urea excretion rates that the proportion of urea removed is quite variable, the variation being due to back diffusion of different amounts of urea from concentrated solution in the lumen of the tubule. They believe that a certain amount of back diffusion takes place normally, and that this may be increased by tubular damage. The small group in Table I associated with general or urogenital infection lends support to this conception, but would extend it to include back diffusion of creatinine as well at least in pathological conditions. This group showed low clearances and yet a normal number of glomeruli which in histological section did not appear significantly abnormal.

While it is of course possible that the volume of glomerular filtrate may have been reduced in these cases it seems more probable that the cause of the low clearance values lies in toxic damage to tubule cells so that they are no longer able to resist back diffusion of urea or creatinine from the lumen of the tubule into the peritubular capillaries. This is illustrated in the group of patients with pneumonia. Cases 7 and 8 had normal concentrations of blood urea nitrogen and creatinine and normal clearances. Case 9 had a normal blood creatinine and creatinine clearance, but an elevated blood urea nitrogen and a reduced urea clearance. This we would interpret as indicating sufficient damage to tubules to permit an increase in the normal back diffusion of urea, but insufficient to allow

appreciable back diffusion of the normally more concentrated creatinine. Cases 10 and 11 had increase of both urea nitrogen and creatinine, and a reduction in both clearances, but a normal number of glomeruli and adequate blood pressure. Here it seems probable that tubule cells were sufficiently damaged to permit back diffusion of both urea and creatinine. We have no explanation to offer for the presence of this type of damage in some pneumonia patients and not in others.

If this interpretation be correct its bearing on the significance of clearance tests is obvious. They may be used as a measure of the "amount" of functioning renal tissue only in the absence of sepsis or toxic damage to tubules. When these are present, clearance tests indicate the manner in which the kidney eliminates the test substance, but how much of a reduced clearance is due to reduction in amount of functioning renal mass and how much to abnormal behavior of that mass it is impossible to say.

Additional evidence that variation in the proportion of urea removed from the blood occurs is indicated by the effect of drugs (Table II). After

TABLE II
Effect of drugs on creatinine and urea clearance tests

Name	Drug	Urine volume	Creatine clearance		Urea clearance	
		cc per minute	cc per minute	Per cent average normal	cc per minute	Per cent average normal
B	Control—1st hour	4 26	169	114	75 0	100
	Control—2nd hour	4 23	164	111	77 0	103
H	Before pituitrin	11 33	138	93	90 0	120
	After pituitrin	66	113	76	26 85*	50
C	Before pituitrin	8 75	86	58	49 5	66
	After pituitrin	3 17	71	48	24 9	33
S	Before adrenalin	7 70	196	132	75 0	100
	After adrenalin	3 75	139	94	57 5	77
B	Before morphine	4 80	181	122	87 0	116
	After morphine	2 75	143	97	82 7	110
L	Before caffeine	1 91	126	85	32 7*	61
	After caffeine	5 68	158	107	73 5	98

* Standard clearance

the administration of pituitrin, the excretion of urea is diminished relatively more than that of creatinine, while after adrenalin and morphine it is decreased less. After caffeine, there is a greater increase in urea clearance than in creatinine clearance. These experiments were all done in the

morning when MacKay (13) has shown that variations in clearances are least. Fluid intake was constant, the drugs given hypodermically five minutes before the end of the control hour. While there is nothing in the simple comparison of the two tests to indicate that it is the proportion of urea removed from the blood that varies, rather than creatinine, the relatively constant rate of creatinine excretion independent of urine volume, and the variation in the rate of urea excretion with urine volume make it seem more probable that there is greater variation in the proportion of urea removed than of creatinine. If the creatinine clearance represents the volume of glomerular filtrate, it must indicate that variations in the volume of filtrate are of less importance in determining the final volume of urine than are differences in the volume of fluid reabsorbed by the tubules.

SUMMARY

The results of creatinine and urea clearance tests of kidney function have been compared with estimates of the number of glomeruli in one kidney after postmortem injection. In arteriosclerotic Bright's disease and probably in chronic hemorrhagic Bright's disease reduction in clearance tests is associated with decrease in the number of injected glomeruli. In pneumonia and pyelonephritis, the clearances may be greatly reduced in the presence of a normal number of glomeruli. It is suggested that this may be due to back diffusion of the test substance through damaged tubule cells.

We are indebted to Dr Allan R. Moritz for the histological examination of the kidneys and for the differential counts of injected and uninjected glomeruli.

BIBLIOGRAPHY

- 1 Moller, E. McIntosh, J. F. and Van Slyke, D. D. *J. Clin. Invest.* 1928 vi 427. Studies of Urea Excretion. II. Relationship Between Urine Volume and the Rate of Urea Excretion by Normal Adults.
- 2 Van Slyke, D. D., Stillman, E., Moller, E., Ehrich, W., McIntosh, J. F., Leiter, L., MacKay, E. M., Hannon, R. R., Moore, N. S. and Johnston, C. *Medicine* 1930 ix 257. Observations on the Courses of Different Types of Bright's Disease and on the Resultant Changes in Renal Anatomy.
- 3 Addison, T. and Oliver, J. *The Renal Lesion in Bright's Disease*. Paul B. Hoeber, Inc. New York, 1931.
- 4 Holten, C. and Rehberg, P. B. *Acta med. Scandinavica* 1931 lxxv 479. Studies on the Pathological Function of the Kidneys in Renal Disease Especially Bright's Disease. I.
- 5 Shannon, J. A., Jolliffe, N. and Smith, H. W. *Am. J. Physiol.* 1932 cii 534. The Excretion of Urine in the Dog. VI. The Filtration and Secretion of Exogenous Creatinine.
- 6 Hayman, J. M. Jr., Halsted, J. A. and Seyler, L. L. *J. Clin. Invest.* 1933 xii 861. A Comparison of the Creatinine and Urea Clearance Tests of Kidney Function.

- 7 Kunkel P A Jr Bull Johns Hopkins Hosp 1930 xlvii 285 The Number and Size of the Glomeruli in the Kidney of Several Mammals
- 8 Fisher R A Statistical Methods for Research Workers Oliver and Boyd Edinburgh 1928 2d ed
- 9 Hayman J M Jr and Starr J Jr J Exper Med 1925 xlii 641 Experiments on the Glomerular Distribution of Blood in the Mammalian Kidney
- 10 Moore R A Anat Rec 1931 xlviii 153 The Total Number of Glomeruli in the Normal Human Kidney
- 11 Vamtrup B Am J Anat 1928 xli 123 On the Number Shape Structure and Surface Area of the Glomeruli in the Kidneys of Man and Mammals
- 12 Hayman J M Jr J Clin Invest 1929 viii 89 Experiments on the Patency of the Blood Vessels of Nephritic Kidneys Obtained at Autopsy
- 13 Mackay L M J Clin Invest 1929 vi 505 Studies of Urea Excretion V The Diurnal Variation of Urea Excretion in Normal Individuals and Patients with Bright's Disease

SERUM TREATMENT OF HEMOLYTIC STREPTOCOCCUS PNEUMONIA

By HAROLD L. AMOSS AND FRILE B. CRAVEN JR.

(From the Department of Medicine, Duke University Hospital and Medical School, Durham)

(Received for publication May 9, 1933)

During the several outbreaks of respiratory disease occurring during the colder months of the year the chance simultaneous occurrence of streptococcus tonsillitis not infrequently results in hemolytic streptococcus lobular pneumonia. Many of the cases of pneumonia seem to be secondary in the sense that the symptoms of an acute infectious disease such as influenza, measles, sinusitis, etc. are present for a few days before the onset of pneumonia, whereas in a smaller number there occurs a sudden onset of lobular pneumonia usually with pleurisy without any evidence of a preceding infection. The seriousness of both types of hemolytic streptococcus pneumonia with the high mortality and frequent occurrence of empyema is well known. The accepted method of treatment is supportive and symptomatic with surgical intervention whenever empyema occurs. The only hope of successful treatment lies in the use of specific agents. Since certain other streptococcal infections such as scarlet fever and erysipelas have apparently improved under the administration of specific antiserum, it is logical to apply these methods to the streptococcal pneumonias. At least one difficulty comes to mind immediately, viz. the problem of preparing a serum of sufficient polyvalency to be generally applicable. The pathogenic hemolytic streptococci vary greatly in their antigenic values. Even the strains isolated from cases of erysipelas and cellulitis show considerable differences not only immunologically but in their fermentative reactions.¹ However, these strains by their breadth of immunological reaction are apparently interrelated and it has been found possible by means of the Lancefield precipitation test to select nine strains for the production of a polyvalent serum sufficiently broad to cover all the strains thus far recovered from cases of erysipelas and cellulitis. This assumption of coverage is based on the Lancefield (1) M factor precipitation reaction in monovalent rabbit sera prepared against each of the nine strains, on the neutralization of the toxin, on protection tests in mice and on clinical response in human cases.

¹ Unpublished experiments of Eleanor A. Bliss.

It has not been found possible to obtain the precipitation reaction with serum from immunized horses.

fair number of white cells as well as albumin although the latter fell to a faint trace before discharge

She was discharged March 19 1930, six weeks after admission

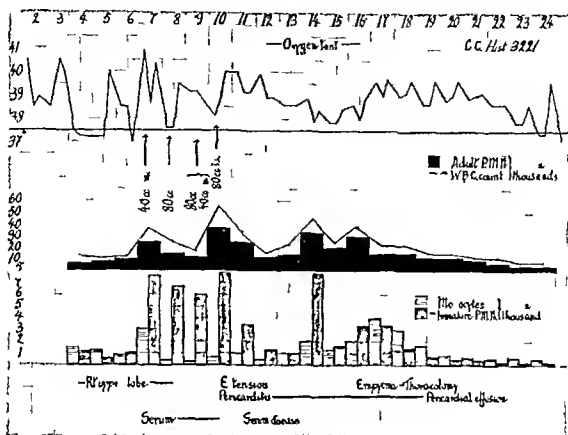


CHART I CASE II

Case II C C male aged twenty two years was well until February 2 1931 when he had a sudden attack of high fever headache weakness nausea and vomiting and was admitted to the infirmary. Recovery was uneventful and he was discharged on February 7th. He was extremely weak and had severe night sweats but returned to school and work on February 12th. One week later he was readmitted to the infirmary with German measles and remained four days. He did not return to work until February 24th. On the evening of February 28th there was a sudden onset of high fever accompanied by aching and he was readmitted to the infirmary on March 1st. For three days the temperature varied from 37° to 40.5° C with two chills each day. On March 5th he was admitted to the hospital with signs of lobular pneumonia in the right upper lobe. The sputum culture showed innumerable colonies of β hemolytic streptococci. The temperature fluctuated between 37° and 41° C. On the sixth day after onset the patient had two chills, seemed much worse and complained of severe pain in the right side of his chest. In the afternoon 40 cc of concentrated erysipelas serum were injected intramuscularly and in the afternoon of the seventh day 80 cc of the unconcentrated serum were given intravenously. These doses were repeated on the eighth day and the intravenous injection repeated again on the tenth day when there were signs of extension to the right lower lobe. During the four days of serum treatment the immature polymorphonuclears increased but decreased on the eleventh day. The fever became continuous instead of remittent. On the twelfth day there were signs of pericarditis and on account of the extreme cyanosis the patient was placed in the oxygen tent for four days. Fluid was detected in the right pleural cavity.

The first opportunity to apply this serum in the treatment of streptococcus pneumonia came before any immunological tests were carried out. But such tests coincidently with treatment were carried out showing that the polyvalent serum possesses antibodies for the particular strain involved.

The purpose of this report is to record the details of the treatment of eight successive cases⁴ of β -hemolytic streptococcus pneumonia all of whom recovered.

Case I. I. D., female, aged twenty one years, was admitted February 14, 1930 complaining of chills, fever and swollen cervical lymph nodes of nine days' duration. Past history: Frequent tonsillitis before tonsillectomy twelve years ago and a rather severe sinusitis one and a half years ago.

The illness for which she was admitted began simply enough as a sore throat and head cold with rather marked swelling of the lymph nodes and later fever.

For a week the temperature was normal every morning, reaching 39.5° and 40° C at night with profuse sweats and later with pain over the right side of the face. On admission she appeared moderately ill and showed little except the red pharynx, the tenderness over the right maxillary frontal sinus and general moderate lymph node enlargement. Her temperature was 39.7° C, pulse 138, blood pressure 130 systolic and 80 diastolic, her leukocytes were 17,600 with 54 per cent polymorphonuclears. The urine showed red and white cells and 2+ albumin.

At this time she was found to have an acute right maxillary sinusitis and a mild acute nephritis. Despite puncture of the intrum four days later her temperature continued to fluctuate widely from 36.5° to 40.5° C in the course of six hours. The urine contained red cells and albumin and the nonprotein nitrogen rose to 48 mgm per cent. β -hemolytic streptococci were grown from the intrum washings. On the twenty-second day after onset she developed signs of pneumonia of the right upper lobe. Cultures and mouse inoculation of the washed sputum showed predominance of β -hemolytic streptococci which by the Lancefield method yielded a specific soluble substance precipitated by monovalent antierysipelas serum. She was given two intravenous injections of anti-erysipelas serum, first 25 cc and then 50 cc. Following this the temperature fell to normal and the excretion of urine rose from 560 cc per day (average for seven days) to 2340 cc the following day and continued at an average of 1460 cc per day.

Her course was then uneventful except for two moderate attacks of serum sickness. She began to gain in weight and strength. Her red count and hemoglobin began to return to normal. Her sinuses cleared and showed no further streptococci on culture although her pharynx continued to harbor a decreasing number of these organisms. On the day of discharge a culture was negative.

The only abnormality was in the urine. The kidney function tests were normal but there were continuously present great numbers of red cells and a

² Polyvalent anti-streptococcus hemolyticus serum (unconcentrated) and its concentrated product (erysipelas antitoxin) prepared by H. K. Mulford Company using nine selected strains. In two cases streptococcus antitoxin prepared by the New York State Laboratory was used in addition.

⁴ Abstracts of the first five cases treated as presented before the Association of American Physicians in 1931 by Amos, H. I., Persons, F. I., and H. G. O. Pruss, O. C.

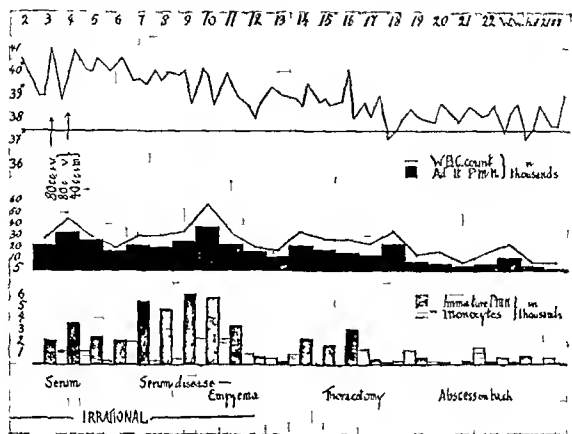


CHART III CASE IV

Case IV W W male aged fifteen years about January 15 1931 experienced a soreness in his throat and the following day was admitted to the infirmary where he was placed in the same room with several patients ill with influenza. About January 30th he had a chill and became very ill. Signs of pneumonia over the entire right side of the chest appeared on February 1st and he was transferred to the Medical Service. By February 2 1931 the sputum contained pus and many β hemolytic streptococci. The x ray showed diffuse peribronchial reaction throughout the right lobes thickened pleura and a shadow obscuring the outer portion of the diaphragm. The patient was irrational the temperature varied from 39° to 41° C and the blood pressure was 80 systolic and 40 diastolic. Eighty cubic centimeters of erysipelas antistreptococcus serum were given intravenously and the treatment repeated the following day with 40 cc of the concentrated serum intramuscularly. The temperature which had fluctuated markedly began to be continuously high and gradually to recede. Serum disease appeared on the seventh day and the immature polymorpho nuclears increased. One hundred cc of fluid were withdrawn from the right pleural cavity on the tenth day but tidal irrigation was not begun until the fifteenth day. Cultures of fluid showed heavy growth of β hemolytic streptococci. The Schilling count shifted to the right and there was a gradual and complete recovery. The patient was discharged well on April 4 1931.

Cases treated by intrapleural injection of serum

In three of the four cases already described empyema developed requiring long drainage. In the two cases to be described the serum was injected into the pleural cavity with a twofold purpose (a) as a mechanical method of affording relief from severe pleurisy and (b) to prevent if possible empyema. In these cases the intravenous and intramuscular injections were also given.

on the eighteenth day. One liter of pus was evacuated and tidal drainage continued. Culture of the empyema fluid showed heavy infection with β -hemolytic streptococci. On the nineteenth day the patient expectorated about 200 cc. of sputum and the signs of pericardial effusion disappeared within two days. On the twenty-fourth day the roentgen ray showed signs of cavitation in the right upper lobe and there was continuous expectoration of pus which contained many spirochetes and β -hemolytic streptococci. After postural drainage and the intravenous injection of near-phenamine the lung abscess gradually healed and the patient gradually improved. He was discharged well on September 30, 1931.

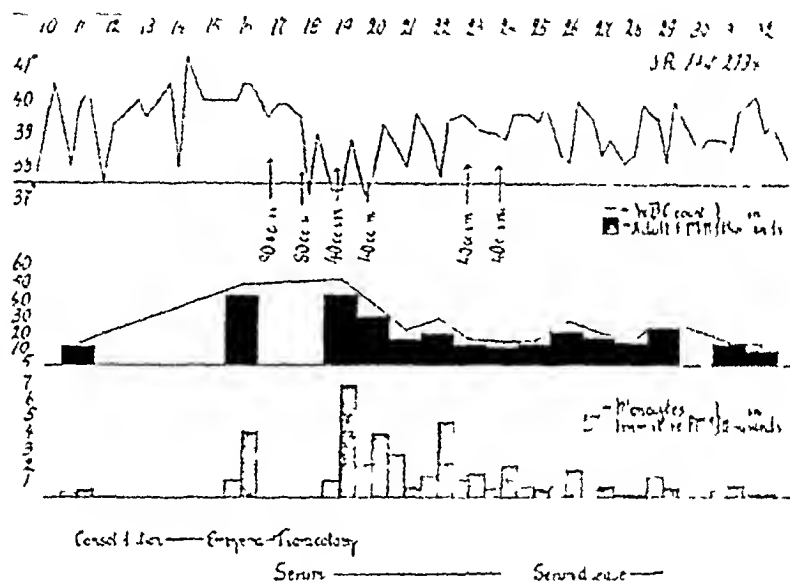


CHART II CASE III

Case III. 1 R male aged sixteen years was admitted January 27, 1931 from the nursery on the tenth day of a very severe laryngitis with a high fever and unproductive cough. On the eleventh day there were signs of consolidation in the left lower lobe. On the thirteenth day there were signs of fluid at the left base and cultures of the sputum taken on this date showed numerous β -hemolytic streptococci. On the sixteenth day there was a rise in the immature polymorphonuclear cells in the blood and fluid containing large numbers of pus cells (culture positive for β -hemolytic streptococci) was removed from the pleural cavity. On the seventeenth day the patient became much worse. On this day 80 cc of crystalline anti-streptococcus serum were given intravenously and the dose repeated the following day and tidal drainage of the pleural cavity was begun. On the succeeding six days there were given intravenous injections of 40 cc each of the concentrated serum intramuscularly. The number of immature polymorphonuclear cells began to recede on the tenth day and the patient began to improve. Convalescence was slow but the patient was discharged well on May 15, 1931.

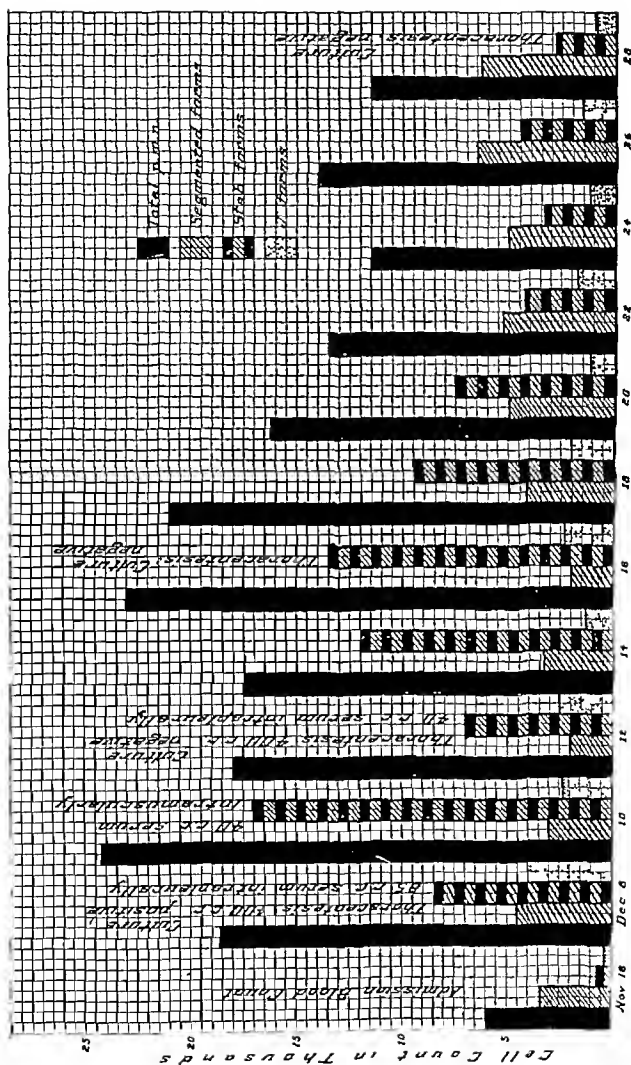


CHART V C1SF VI TOTAL AND SCHILLING COUNTS OF WHITE BLOOD CELLS

STREPTOCOCCUS PNEUMONIAE

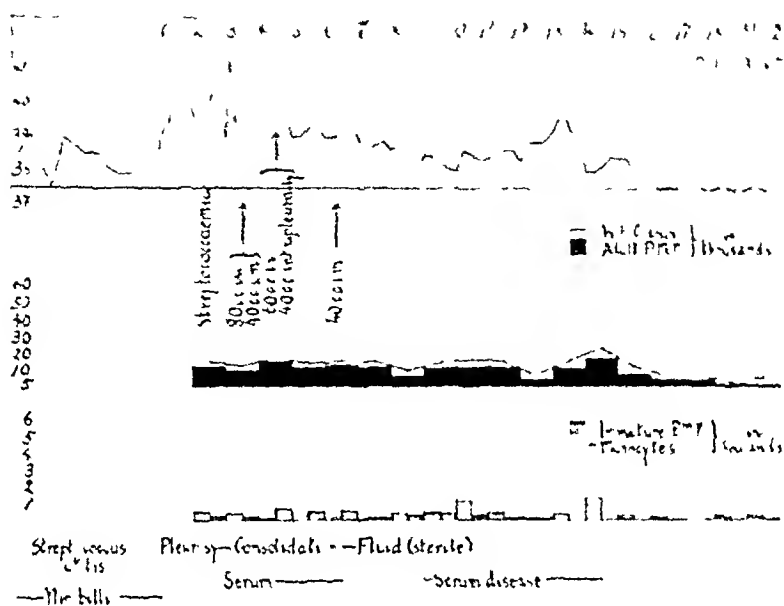


CHART IV CASE V

As evidence that the effect of the serum is a result of specific action rather than a non specific provocation of reticuloendothelial cells (2) two cases are described below in which the pleural fluid contained in addition to the β hemolytic streptococcus a strain of pneumococcus Group IV. After adequate doses of the serum streptococci were no longer present but the pneumococci endured.

Case VII A six year old colored female was admitted to the Pediatric Ward⁵ on January 11, 1933 on the seventh day of lobular pneumonia and with signs of fluid in the left pleural cavity. The temperature was 40.5° C and white blood cells 20,500 per cubic millimeter. Sputum yielded pure culture of β hemolytic streptococci and the blood culture showed less than one colony per cc of the same strain. Thoracentesis immediately after admission yielded a small quantity of sanguineous fluid which on culture, had a heavy growth of β hemolytic streptococci. The patient grew progressively worse. On January 15th and 16th 15 and 40 cc of fluid respectively were withdrawn and cultures were heavily positive. On January 17th following aspiration of 30 cc of fluid 10 cc of erysipelas antitoxin were instilled into the pleural cavity. There was no reaction. On January 20, 1933 40 cc of erysipelas antitoxin were injected intrapleurally following aspiration of 280 cc of pleural exudate. Culture of this aspirated fluid again yielded β hemolytic streptococci. Cultures of fluid removed on January 25th and 27th were negative for hemolytic streptococci but gave a heavy growth of pneumococcus Group IV. Before serum was given the temperature reached daily peaks of 39° to 40.6° C and the white blood count 25,000. Following the serum injections the white blood count dropped rapidly to 12,000 and the peaks of fever to 38.5° to 37.2° C. After thoracenteses on January 25th and January 27th (yielding Group IV pneumococci) the temperature again rose to 40.6° C.

Thoracotomy for the purpose of tidal irrigation was performed on January 30, 1933. Pus obtained at operation gave a pure culture of pneumococcus Group IV.

Sputum culture now gave a mixed growth of pneumococcus Group IV, staphylococcus aureus and β hemolytic streptococci. Blood cultures remained sterile.

Four days after operation the temperature fell to 37.6° C and remained normal until March 13th when the drainage tube was removed. There was an immediate rise in temperature to 39.3° C and the child became quite ill again. Drainage was re-instituted and the temperature came to normal after six days. At discharge March 24, 1933 the thoracotomy wound had healed and the child was discharged in excellent condition.

Case VIII Colored male, aged four years, brother of patient described under Case VII, admitted on January 11, 1933 on the fifth day of lobular pneumonia of left lung with pleurisy, thickened pleura and early empyema. Temperature 40.2° C, white blood cells 11,600, sputum yielded a heavy growth of β hemolytic streptococci and the blood culture showed less than one colony per cc of the same organism. Thoracentesis on the day of admission yielded a small quantity of sanguineous fluid which on culture had a heavy growth of β hemolytic streptococci.

⁵ We are greatly indebted to Dr. W. C. Davison, Professor of Pediatrics, for permission to report Cases VII and VIII and to Dr. Jean D. Craven, Resident in Pediatrics, for the clinical observations on them.

lized. The temperature became normal on February 15th and there was an ineventful recovery save for severe serum sickness. The patient was discharged well on March 14th.

Case II. White male, aged forty-eight years, admitted on February 1, 1932, with the diagnoses of pernicious anemia and subacute combined sclerosis of spinal cord. He was treated with liver and liver extract and was discharged improved on February 14th.

He continued to take liver extract irregularly and was readmitted on November 18, 1932, with more advanced spinal cord changes and a neurological bladder. There had been only a moderate diminution in the erythrocyte count and hemoglobin.

On the eleventh day after admission his temperature rose to 39°C and the leukocyte count to 14,500. Examination disclosed nothing to account for the fever. The irregular fever rising to 38.5° to 39.5°C daily continued and on December 7th the patient complained of severe pain in the right flank and along the lower right anterior costal margin. Respirations were shallow and there was diminished excursion on the right. Tactile fremitus was moderately increased from the angle of the scapula to the right base. The percussion note was slightly impaired and a loud rough friction rub obscured the breath sounds. On the following morning the breath sounds were tubular and the friction rub was still present. The patient was acutely ill and was very uncomfortable. Culture of the sputum showed heavy growth of β hemolytic streptococci. With the appearance of the fluid at the right base the audible rub disappeared but the severe pain endured. The blood culture taken on December 8th showed one colony of β -hemolytic streptococci per cubic centimeter. On December 9th from the right pleural cavity, 400 cc of thin bloody fluid were withdrawn which on culture yielded a heavy growth of β hemolytic streptococci. Following thoracentesis 85 cc of concentrated crystalline streptococcus antitoxin diluted with 85 cc of physiological saline were injected directly into the pleural cavity without immediate reaction. There was no increase in the pain.

On December 10th 40 cc of the antitoxin were given intramuscularly. On December 11th 500 cc of pleural fluid were again withdrawn and 40 cc of concentrated streptococcus antitoxin injected. The fluid withdrawn was no longer bloody and cultures remained sterile. The leukocyte count of 37,000 on the day before injection of serum fell to 24,000 on the following day and to 17,000 on the third day (see Chart V). The fever which during the height of the disease ranged between 38.5 and 40°C now fluctuated between 38 and 38.8°C . The patient looked and felt much improved although signs of considerable pleural effusion remained. The irregular fever continued. Thoracenteses on December 17th and 29th and January 13, 1933, yielded quantities of fluid from 40 to 500 cc. All were cultured and remained sterile. The temperature gradually fell to normal during the five weeks following serum injection. The patient was discharged on January 26, 1933, having been afebrile for two weeks but with evidence of a small amount of encapsulated fluid along the right lateral chest wall.

Specific action of the serum

In Case V it is possible that the intrapleural instillation of serum prevented infection of the pleural fluid by streptococci and in Case VI the serum apparently assisted within twenty-eight hours in the removal of the previously established

As evidence that the effect of the serum is a result of specific action rather than a non specific provocation of reticuloendothelial cells (2) two cases are described below in which the pleural fluid contained in addition to the β -hemolytic streptococcus a strain of pneumococcus Group IV. After adequate doses of the serum streptococci were no longer present but the pneumococci endured.

Case VII A six year old colored female was admitted to the Pediatric Ward on January 11, 1933 on the seventh day of lobular pneumonia and with signs of fluid in the left pleural cavity. The temperature was 40.5°C and white blood cells 20,500 per cubic millimeter. Sputum yielded pure culture of β hemolytic streptococci and the blood culture showed less than one colony per cc of the same strain. Thoracentesis immediately after admission yielded a small quantity of sanguineous fluid which on culture had a heavy growth of β hemolytic streptococci. The patient grew progressively worse. On January 15th and 16th, 15 and 40 cc of fluid respectively were withdrawn and cultures were heavily positive. On January 17th following aspiration of 30 cc of fluid 10 cc of erysipelas antitoxin were instilled into the pleural cavity. There was no reaction. On January 20, 1933 40 cc of erysipelas antitoxin were injected intrapleurally following aspiration of 280 cc of pleural exudate. Culture of this aspirated fluid again yielded β hemolytic streptococci. Cultures of fluid removed on January 25th and 27th were negative for hemolytic streptococci but gave a heavy growth of pneumococcus Group IV. Before serum was given the temperature reached daily peaks of 39 to 40.6°C and the white blood count 25,000. Following the serum injections the white blood count dropped rapidly to 12,000 and the peaks of fever to 38.5° to 37.2°C . After thoracenteses on January 25th and January 27th (yielding Group IV pneumococci) the temperature again rose to 40.6°C .

Thoracotomy for the purpose of tidal irrigation was performed on January 30, 1933. Pus obtained at operation gave a pure culture of pneumococcus Group IV.

Sputum culture now gave a mixed growth of pneumococcus Group IV, staphylococcus aureus and β hemolytic streptococci. Blood cultures remained sterile.

Four days after operation the temperature fell to 37.6°C and remained normal until March 13th when the drainage tube was removed. There was an immediate rise in temperature to 39.3°C and the child became quite ill again. Drainage was re-instituted and the temperature came to normal after six days. At discharge, March 24, 1933 the thoracotomy wound had healed and the child was discharged in excellent condition.

Case VIII Colored male aged four years brother of patient described under Case VII admitted on January 11, 1933 on the fifth day of lobular pneu-

*Skin neutralization tests*⁶

The streptallergen from four strains (Cases II to V) gave a positive skin reaction in goats. The addition of 0.1 cc. of serum from a goat immunized with the New York 5 scarlet strain of β hemolytic streptococcus prevented any reaction in the presence of 10 skin test doses of the streptallergens.

*Protection tests*⁷

The virulence of four of the strains was increased by mouse passage until the minimum lethal dose became 0.5 cc. of 10^{-7} dilution.

The protection tests were made by injecting intraperitoneally 0.2 cc. of the anti serum diluted to 0.5 cc. with isotonic salt solution and simultaneously but separately 0.5 cc. of the dilution of the eighteen hour broth culture of the organism. Three mice were used for each dilution and a virulence control and normal horse serum control were injected in each series.

The results of the protection tests were as follows:

Case I Not tested

Case II Virulence 10^{-6} Mulford's concentrated protected against 10^{-7} unconcentrated result inconsistent, New York State no protection.

Case III Virulence 10^{-6} , Mulford's concentrated protected against 10^{-5} unconcentrated result inconsistent. New York State no protection.

Case IV Virulence 10^{-6} Mulford's concentrated protected 2 out of 3 against 10^{-4} , unconcentrated protected against 10^{-7} , New York State no protection.

Case V Control 2 out of 3 mice died after injection of 10^{-4} dilution plus normal horse serum. Mulford's concentrated streptococcus antitoxin protected all mice against 10^{-3} . New York State antistreptococcus serum protected 2 mice out of 3 against 10^{-7} .

Cases VI, VII and VIII strains *avirulent* for mice.

Protection tests with convalescent serum from the patients have not yet been made.

DISCUSSION

The patients treated with serum were critically ill. With the high fever, cherry cymosis, hypotension, distension and evidences of a rapidly spreading lesion the prognosis was very grave. In Case I the administration of the serum was followed by a marked increase of urinary secretion and quick disappearance of fever with a definite improvement in the

⁶ These tests were made by Miss Ruth Wheeler of the New York State Laboratory of Hygiene to whom we are greatly indebted for her cooperation.

⁷ Method of Mr. C. Roos, Glenolden, Pa.

mouse protection tests suggest a rational immunologic basis for specific serum treatment

BIBLIOGRAPHY

- 1 Lancefield Rebecca C J Exper Med 1928 *ibid* 91 and *ibidem* 469 The Antigenic Complex of Streptococcus Hemolyticus 1 Demonstration of a Type Specific Substance in Extracts of Streptococcus Hemolyticus
- 2 Gay F P and Clark A R J Exper Med 1930 *ibid* 95 Enhanced Passive Immunity to Streptococcus Infection in Rabbits

CYSTINE CONTENT OF FINGER NAILS IN PELLAGRA¹

By SHELDON A. PAYNE AND WILLIAM A. PERLZWEIG

(From the Department of Biochemistry, Duke University School of Medicine and Hospital, Durham)

(Received for publication May 29 1933)

An abnormality in sulfur metabolism in pellagra was first demonstrated by Camurri (1) and Myers and Fine (2). Indicanuria (3, 4), a decreased excretion of thiocyanate (5), and an increase in the excretion of absolute amounts of ethereal sulphates in the urine (2) of pellagrins have been observed. A reduction of sulfur in brain and spinal cord of five individuals succumbing with pellagra was reported by Koch and Voegtlin (6). The total amount and per cent cystine of rat hair decreases when these animals are fed diets deficient in vitamin G (7, 8).

It has been suggested (9) that the relatively high concentration of cystine in wool and hair is of physiologic importance in the protection of the organism against harmful effects of prolonged exposure to sunlight. The protecting effect of cystine solutions to paramecia exposed to ultraviolet rays was demonstrated by Harris and Hoyt (10) and experiments of Ward (11) seem to indicate that although several amino acids give marked general absorption bands, cystine is the only amino acid that has any marked absorption in the ultraviolet region of solar light. The high concentration of cystine in epidermal tissues of higher forms of animal life (12, 13) a content greater than that of any other tissue, suggests a possible specific protective function in this tissue. In the hope that we might find more experimental evidence for the clinical observation (14, 15) that skin lesions in pellagra indicate a light sensitive condition 40 cases of pellagra and 61 subjects without evidence of pellagra have been studied. Because of the technical difficulties in obtaining representative samples of skin we concluded that finger nails gave more uniform data from which to judge cystine content in epithelial tissue.

METHODS

In reviewing the available data concerning the sulfur and cystine content of the common keratins one is impressed with the wide variation of the results in the hands of different observers. Much of the data is open to criticism in that no mention is made concerning the method of preliminary treatment and variations in this may account for many of the discrepancies. Our preliminary work showed that if nails are thoroughly cleaned and the technique of drying standardized there is little variation in cystine in nails from various fingers of the same individual. However in spite of an attempt to subject each sample of

¹ This investigation was aided in part by a grant from the Pellagra Fund donated by the Lederle Laboratories, Inc.

of 97 to 117 per cent Sullivan and Hess (23), in 1932, reported cystine content of the finger nails of normal individuals to vary from 11 to 13 per cent Since the early methods involved the isolation of cystine from a hydrolysate and determination of precipitated sulfate, one would expect higher values in a colorimetric method such as Sullivan's

In our analyses of finger nails from 36 healthy young adults, chiefly medical students and laboratory workers, Figure 1, A and Table I, cystine

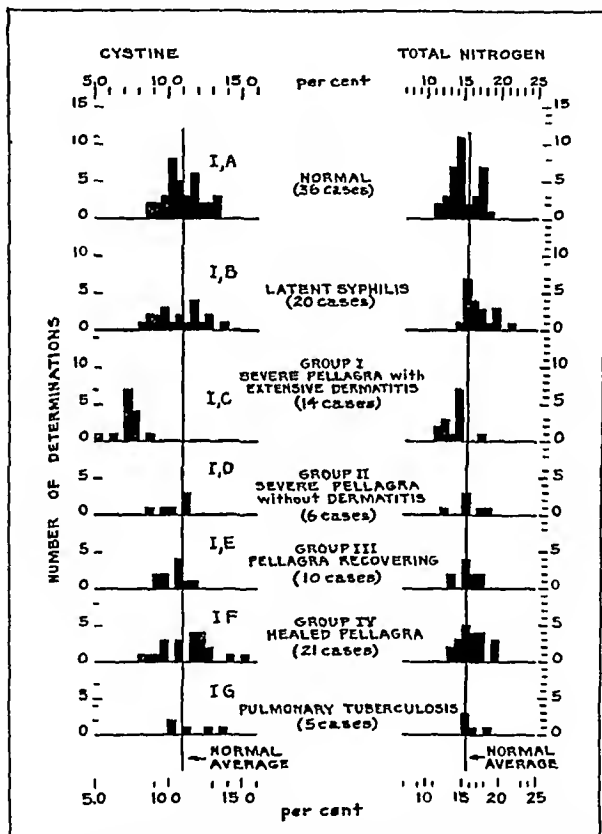


FIG 1 HISTOGRAMS FOR CYSTINE AND TOTAL NITROGEN CONTENT OF THE FINGER NAILS IN NORMAL SUBJECTS AND IN PATIENTS WITH PELLAGRA SYPHILIS AND TUBERCULOSIS

Each square represents a single determination

nail parings to standard conditions of cleaning and drying it was realized that some variation in the rate of loss of water from different samples was unavoidable. Therefore the total nitrogen content (representing protein) and the N:S ratio were determined in a further effort to minimize the errors inherent in a series of determinations based on the dry weight of biological material.

Finger nail parings were soaked in water for 24 hours, cuticle and dirt removed by scraping and after washing in running water dried in an oven for 24 hours at 90° C. Cystine was determined by a modification of the Sullivan method (16). From 30 to 60 mgm clean dry finger nail parings and 5 cc of 20 per cent hydrochloric acid were placed in a 50 cc acetylation flask with a ground glass fitted reflux condenser. The flask was placed in a cold mineral oil bath and the bath brought to 120° C at which heating was continued for 12 hours. The hydrolysate was transferred to a 25 cc volumetric flask and the acetylation flask rinsed with 5 cc of 0.1 N hydrochloric acid. The hydrolysate was treated drop-wise with 20 per cent sodium hydroxide until greenish to bromphenol blue, i.e., about pH 3.5. Dilution was made to 25 cc with addition of 0.1 N hydrochloric acid. Colorimetric estimation of cystine proceeded according to Sullivan's method. Color matching is facilitated by the use of a yellow light filter glass disc devised for the Folin and Malmros (17) micro method for blood sugar determination.²

Total nitrogen of finger nails was determined by the Kochi-McMeekin method (18) on the above hydrolysate.

Because of the slow and variable growth of the finger nail only cases with chronic disease have been considered and only stable conditions of at least two months duration have been included in the data. Thirty-three of the pellagrins were negro patients at the State Hospital at Goldsboro, North Carolina,³ and were carefully followed for months. Several had experienced "recurrent" pellagra for years and had been observed at the institution over long periods of time. All gave histories of faulty dietaries for months, and three mental cases were observed to develop symptoms during self-inflicted periods of starvation. Treatment in all cases was chiefly dietary and followed the suggestions of Goldberger et al (19, 20). High vitamin foods, especially milk and fresh meats, fruits, and vegetables, were given in abundance. Since many of the pellagrins were known to have syphilis on the basis of history of infection or positive blood Wassermann tests, data are presented from a control group of 20 cases with latent syphilis. Also since emaciation is common among pellagrins five malnourished cases of pulmonary tuberculosis are included.

Cystine content of normal finger nails

Only a few values for the cystine or sulfur content of normal human finger nails are available. The early work of Mulder (21), in 1847, showed human nail to contain 2.8 per cent sulfur corresponding to 10.5 per cent cystine. Buchholz (13), in 1907, pooled the finger nails from 16 cadavers and by a method which determined sulfur by sublimation, estimated cystine content to be 5.15 per cent. Langecker (22), in 1921 reported values for sulfur in finger nails from six patients with various diseases. His values range from 2.59 to 3.12 per cent corresponding to estimated cystine values

² Manufactured by the Klett Manufacturing Company, New York City.

³ The authors are indebted to the Superintendent W. Linville, M.D. and his staff for the opportunity to study and to obtain samples of finger nail from these patients.

TABLE II

Cystine, total nitrogen and N S ratio in finger nails of individual negro cases of severe pellagra with extensive dermatitis before and after recovery

Case number	Sex	Age	Cystine	Total nitrogen	N S ratio *	Clinical notes
		<i>years</i>	<i>per cent</i>	<i>per cent</i>		
101	F	50	7 04	16 0	8 51	Extensive dermatitis Died 2 weeks later
107	F	60	7 46	15 8	7 94	Severe dermatitis Later recovered
108	F	63	8 55	15 4	6 76	Severe dermatitis Died 4 weeks later
109	F	53	7 52	12 6	6 27	Extensive dermatitis
			11 80	15 7	4 98	4 months after recovery
111	F	32	7 69	13 8	6 74	Severe dermatitis Later recovered
112	F	30	7 50	13 8	6 90	Extensive dermatitis
			10 90	17 9	7 43	6 months after recovery
113	F	29	7 32	13 1	6 71	Severe dermatitis
			8 22	16 3	7 41	3 months after recovery
114	F	46	7 27	12 6	6 50	Severe dermatitis
			10 83	13 7	4 74	5 months after recovery
115	F	45	8 00	15 4	7 20	Extensive dermatitis Died 9 weeks later
118	M	17	7 09	15 0	7 98	Severe dermatitis
			10 72	14 3	5 00	6 months after recovery
119	M	70	14 49	19 2	4 95	3 months before onset of symptoms
120	M	32	7 85	14 5	6 90	Severe dermatitis
			5 03	15 1	11 28	Most severe dermatitis
			9 53	17 8	7 01	4 months after slow improvement
127	M	27	6 15	18 1	11 01	Very severe dermatitis Later recovered
140	M	40	7 24	15 7	8 14	Severe dermatitis Later recovered

14 cases severe dermatitis

Minimum	5 03	12 6	6 27
Maximum	8 55	18 1	11 27
Average	7 26	14 8	7 63

5 cases following recovery

Minimum	8 22	13 7	4 74
Maximum	11 08	17 9	7 43
Average	10 49	15 6	5 91

* S calculated from cystine

TABLE I

Summary of cystine, total nitrogen and N S ratio in finger nails

	Num- ber of Cases	Cystine			Total nitrogen			N S Ratio*		
		Mini- mum	Maxi- mum	Ave- rage	Mini- mum	Maxi- mum	Ave- age	Mini- mum	Maxi- mum	Ave- age
Normal	36	8.77 <i>per cent</i>	13.38 <i>per cent</i>	10.97 <i>per cent</i>	12.6 <i>per cent</i>	18.7 <i>per cent</i>	15.6 <i>per cent</i>	4.61 <i>per cent</i>	5.92 <i>per cent</i>	5.40 <i>per cent</i>
Pellagra										
Group I										
Severe with der- matitis	14	5.03	8.55	7.26	12.6	18.1	14.8	6.27	11.27	7.63
Group II										
Severe without der- matitis	6	8.93	11.98	10.61	12.6	18.3	16.2	4.71	6.17	5.71
Group III										
Healing	10	9.26	11.93	10.47	13.5	17.6	15.5	1.57	6.16	5.56
Group IV										
Cured	21	8.22	15.42	11.32	13.6	19.8	16.3	4.53	7.13	5.40
Syphilis	20	8.20	13.55	10.64	14.1	21.9	16.9	4.81	7.11	5.95
Tuberculosis	5	10.28	13.64	11.66	15.1	18.8	16.3	4.63	5.91	5.25

* S calculated from cystine

varies from 8.77 to 13.38 per cent with an average of 10.97 per cent and total nitrogen varies from 12.6 to 18.7 per cent with average of 15.8 per cent. The N S ratio averages 5.40 and remains relatively constant with extremes of 4.61 and 5.92. The greater range of cystine in our group compared to that of Sullivan, using essentially the same method, may possibly be explained on the basis of variation in selection of individuals for the "normal" group.

Cystine content of finger nails in pellagra, syphilis, and pulmonary tuberculosis

The pellagrins are arranged into four arbitrary groups: Group I, severe pellagra with extensive dermatitis; Group II, severe pellagra without dermatitis; Group III, pellagra during recovery; and Group IV, completely healed pellagra. An exfoliative dermatitis of the hands, arms, and feet and in most cases of the legs and thighs had been apparent in the 14 cases in Group I for at least two months. All cases were extremely ill at the time of the determination and Cases 101, 108, and 115 (see Table II) died with inanition soon after the observation. A stomatitis was common to all and several had in addition diarrhea, psychosis, or dementia. In this group (see Figure 1, C and Table I) cystine is greatly reduced averaging 7.26 per cent. The highest result, 8.55 per cent, falls below the lower limit of the normal range. In Cases 120 and 127 (see Table I) in which

lagra during which time the patient remained on an adequate diet containing an abundance of all vitamins. Thirteen had recovered from severe pellagra with extensive dermatitis, four from severe pellagra of the gastrointestinal or central nervous system type in which dermatitis had been absent or played a minor role, and four from mild diarrhea and slight dermatitis. In this group (see Figure 1 *F* and Table I) cystine varies from 8.22 to 15.42 per cent. Two results, 14.49 per cent and 15.42 per cent, lie above the upper normal limit. Total nitrogen varies from 13.6 to 19.8 per cent also showing a greater variation than normal in the upper limit. Both the average cystine and total nitrogen approximate the average of the normal group.

The group of 20 luetic consists of patients treated for at least two months at the syphilis dispensary of the Duke Hospital, and although at the time the analyses were made, none had active lesions, all had a history of luetic infection and the blood Wassermann test had been four positive. The group is representative of widely divergent dietaries as observed in mill hands, farmers, shopkeepers, etc. in the pellagra belt. No definite deficiency could be ascribed to any diet although several of the patients were obviously malnourished and many consumed large amounts of corn and fat pork to the exclusion of fruits and other vegetables and meats. Cystine varies more widely in this group (see Figure 1 and Table I) than in the normal, 8.20 to 13.55 per cent, in three cases the result falling below the lower limit of normal and in one case above the upper limit. However, the average for the group 10.64 per cent, approximates the normal average. Total nitrogen tends to be increased varying from 14.1 to 21.9 per cent and averaging 16.9 per cent, thereby increasing the average N/S ratio to 5.95.

The 5 cases of pulmonary tuberculosis gave histories of loss of 20 to 60 pounds of weight during the preceding months and were obviously malnourished, judging by the severe emaciation. Cystine and total nitrogen tend to be above normal in these cases (see Figure 1, *G* and Table I), cystine ranging from 10.28 to 13.64 per cent and averaging 11.66 per cent and total nitrogen from 15.1 to 18.8 per cent and averaging 16.3 per cent. Because of the concomitant rise of both cystine and total nitrogen the N/S ratio values fall within normal limits.

DISCUSSION AND SUMMARY

The foregoing results support the opinion that there is an abnormality of sulfur metabolism in pellagra which is reflected in epithelial tissue and specifically related to the dermatitis of this disease. A study of the data from 36 normals and 20 cases of syphilis shows no constant variation of cystine with difference in sex, race, or age. Since cystine was reduced and N/S ratio increased in the finger nails from all of the 14 cases of pellagra with severe dermatitis, whereas in all cases of pellagra without

dermatitis was most extensive and severe, cystine content is the lowest, 5.03 per cent and 6.15 per cent. A determination three months before the appearance of dermatitis and diarrhea in Case 119 (see Table I) is of particular interest since at that time the cystine content was 11.49 per cent. At this time, because of emaciation, the patient was taking a diet rich in vitamins and calories. Later he refused food and in three months developed a severe dermatitis. Six months after the original observation cystine had decreased to 7.85 per cent, a reduction of 46 per cent. In five cases results were obtained following recovery (see Table II). Cystine had returned to normal limits in all cases and at this time the average cystine, 10.49 per cent, was but slightly below the average for the normal group. Total nitrogen remained practically unchanged varying from 12.6 to 18.1 per cent with an average of 14.8 per cent. The reduction in cystine results in a relative increase in the N : S ratio.

Group II, severe pellagra without dermatitis, consists of six cases: four extremely emaciated, weak, demented individuals suffering from a persistent diarrhea of 15 to 25 stools a day for at least the past two months, and two cases with vicious dementia and mild diarrhea, weakness, and glossitis of seven and of thirty months duration. Although diarrhea was resistant to treatment, all cases recovered after a prolonged improved dietary regime. Skin lesions were observed in only one case and in this case the discoloration and fine desquamation over the feet were so greatly eclipsed by the violent dementia and diarrhea that he is included in this group. However, it is interesting to note that cystine in this case, 8.93 per cent, is the lowest value in the group. All values for cystine and total nitrogen (see Figure 1, D and Table I) fall within normal limits, cystine ranging from 8.93 to 11.98 per cent, and total nitrogen from 12.6 to 18.3 per cent. The average N : S ratio approximates that of the normal group. Determinations after recovery in two cases show no striking change in cystine nor in total nitrogen.

Group III consists of 10 cases all of whom had been recovering for the past two or three months from mild dermatitis of the feet and hands, and stomatitis. Intermittent diarrhea had been common and four cases had developed temporary psychoses. At the time of analysis dermatitis had disappeared and all were much improved. Cystine varied from 9.26 to 11.93 per cent, averaging 10.47 per cent, and total nitrogen 13.5 to 17.6 per cent with an average of 15.5 per cent, all within normal limits (see Figure 1, E and Table I). The N : S ratio averaged 5.56 with extremes of 4.57 and 6.16. Results from three cases following complete recovery showed no change except in one case in which cystine increased from 9.37 per cent to 12.25 per cent, total nitrogen from 15.4 per cent to 16.9 per cent and the N : S ratio from 6.16 to 5.16.

There are 21 cases of healed pellagra in Group IV. In this group analyses were made 3 to 6 months after disappearance of all signs of pel-

lagra during which time the patient remained on an adequate diet containing an abundance of all vitamins. Thirteen had recovered from severe pellagra with extensive dermatitis, four from severe pellagra of the gastrointestinal or central nervous system type in which dermatitis had been absent or played a minor role, and four from mild diarrhea and slight dermatitis. In this group (see Figure 1 *F* and Table I) cystine varies from 8.22 to 15.42 per cent. Two results, 14.49 per cent and 15.42 per cent, lie above the upper normal limit. Total nitrogen varies from 13.6 to 19.8 per cent also showing a greater variation than normal in the upper limit. Both the average cystine and total nitrogen approximate the average of the normal group.

The group of 20 luetics consists of patients treated for at least two months at the syphilis dispensary of the Duke Hospital, and although at the time the analyses were made none had active lesions, all had a history of luetic infection and the blood Wassermann test had been four positive. The group is representative of widely divergent dietaries as observed in mill-hands, farmers, shopkeepers, etc. in the pellagra belt. No definite deficiency could be ascribed to any diet although several of the patients were obviously malnourished and many consumed large amounts of corn and fat pork to the exclusion of fruits and other vegetables and meats. Cystine varies more widely in this group (see Figure 1 and Table I) than in the normal, 8.20 to 13.55 per cent, in three cases the result falling below the lower limit of normal and in one case above the upper limit. However, the average for the group, 10.64 per cent, approximates the normal average. Total nitrogen tends to be increased, varying from 14.1 to 21.9 per cent and averaging 16.9 per cent, thereby increasing the average N:S ratio to 5.95.

The 5 cases of pulmonary tuberculosis gave histories of loss of 20 to 60 pounds of weight during the preceding months and were obviously malnourished, judging by the severe emaciation. Cystine and total nitrogen tend to be above normal in these cases (see Figure 1, *G* and Table I) cystine ranging from 10.28 to 13.64 per cent and averaging 11.66 per cent, and total nitrogen from 15.1 to 18.8 per cent and averaging 16.3 per cent. Because of the concomitant rise of both cystine and total nitrogen the N:S ratio values fall within normal limits.

DISCUSSION AND SUMMARY

The foregoing results support the opinion that there is an abnormality of sulfur metabolism in pellagra which is reflected in epithelial tissue and specifically related to the dermatitis of this disease. A study of the data from 36 normals and 20 cases of syphilis shows no constant variation of cystine with difference in sex, race, or age. Since cystine was reduced and N:S ratio increased in the finger nails from all of the 14 cases of pellagra with severe dermatitis, whereas in all cases of pellagra without

dermatitis cystine and total nitrogen remained approximately normal it is reasonable to assume that the reduction of cystine in finger nail is closely related to the pathological changes in the epidermal tissues in pellagra. This would seem to indicate that a lack of cystine or deficient utilization of this amino acid becomes manifest in the skin lesions of pellagra. On the other hand, such a deficiency of cystine cannot be held responsible for the entire symptom complex of pellagra, since cases of pellagra without dermatitis in whom there is no diminution of cystine in the epidermal tissue, as indicated by our data, are common.

Goldberger and Tanner (24) at one time advanced the theory of a deficiency of amino acids as the probable cause of pellagra and suggested that a deficiency in the supply or utilization of the amino acid, cystine, resulted in pellagra. Support for this theory in explaining the skin lesions of pellagra is given by the work of Harris and of Ward previously cited and by our data. Rough estimations of cystine in the dietary of our patients who developed pellagra failed to show a deficiency in the supply of cystine although a vitamin deficiency was probable. The high cystine values in the finger nails in the group following dietary treatment may indicate greater cystine utilization influenced by adequate vitamin consumption. However, as pointed out by Smith (25), the foods used in the treatment of pellagra contain large amounts of cystine and the increased concentration in healed cases may be due to increased intake of this amino acid *per se*, especially since Beadles et al (26) have demonstrated that the rat's coat of hair becomes heavier when the diet is supplemented with cystine.

Injections of thiosulfate have been advocated and extensively used with apparent benefit in the treatment of pellagra. Sabry (27) reports that the skin lesions of pellagra disappear after a few injections, and that there is marked improvement of diarrhea and of the central nervous system manifestations of this disease after a short course of treatment, consisting of daily intravenous 10 cc injections of 10 per cent solution of sodium thiosulfate. Although Goldberger et al (19, 20) have demonstrated that pellagra may be prevented and cured by supplying the pellagra-preventing factor (vitamin G) in the diet of the pellagrin, and although the thiosulfate treatment is usually combined with an improved dietary regime, in view of the demonstrated alteration in sulfur metabolism, it is likely that thiosulfate is of some benefit to the pellagrin and that some of the rapid improvements observed may be due to this factor directly.

Although we cannot conclude that because of a reduction of cystine in finger nails there must be a reduction of cystine in the skin and therefore an increased sensitiveness to light, it is reasonable to suspect that a reduction of nail cystine is accompanied by a reduction of skin cystine. We contemplate further experimental work to determine the importance of this change in sulfur metabolism and its relation to solar radiation in pellagra.

CONCLUSIONS

1 The cystine content of finger nails was determined in 36 normal subjects in 40 cases of various forms and stages of pellagra and in 25 cases of syphilis and tuberculosis

2 In 14 cases of pellagra with extensive dermatitis there was a marked reduction in the cystine content without any appreciable change in the total protein content of the finger nails. With the subsidence of skin symptoms and improvement in the clinical condition the cystine content returned to normal limits

3 In severe pellagrins without dermatitis (involvement chiefly of the gastro intestinal or central nervous systems) and in partially or completely cured cases of pellagra there were no marked changes in the cystine content of the finger nails

4 Values for cystine content of the finger nails from a group of syphilitics and from a group of tuberculous patients were found to approximate the normal range regardless of the nutritional state of the patient

BIBLIOGRAPHY

- 1 Camurri. Atti del Quarto Congresso Pellagrologico Italiano Udine, 1910 67 Cited by Kahn M and Goodridge F G Sulfur Metabolism Lea and Febiger Co Philadelphia 1926
- 2 Myers, V C and Fine M S Am J Med Sc 1913 cxlv, 705 Metabolism in Pellagra
- 3 Sullivan M Y J Biol Chem 1921 xli Scientific Proceedings p 111 Indian in the Saliva of a Case of Pellagra Complicated with Tuberculosis
- 4 Ridlon J R U S Pub Health Rep 1916 xxxi 1979 Pellagra The Value of the Dietary Treatment of the Disease
- 5 Sullivan M Y and Dawson P R J Biol Chem 1920 xli, 473 Sulfoevanate Content of the Saliva and Urine in Pellagra
- 6 Koehl M L and Voegtlin C U S Hyg Lab Bull 1916 No 103 51 Chemical Changes in the Central Nervous Systems in Pellagra
- 7 Chick H and Roscoe M H Biochem J 1927 xxi 698 On the Composite Nature of the Water soluble B Vitamin
- 8 Goldberger J and Lillie R D U S Pub Health Rep, 1926 xli 1025 A Note on an Experimental Pellagra like Condition in the Albino Rat
- 9 Lewis H B Physiol Rev 1924 iv 394 Sulphur Metabolism
- 10 Harris F I and Hoyt H S Science 1917 xli 318 The Possible Origin of the Toxicity of Ultra violet Light
- 11 Ward I W Biochem J 1923 xvi 898 The Absorption Spectra of Some Amino Acids The Possible Ring Structure of Cystine
- 12 Wilson R H and Lewis H B J Biol Chem 1927 lxxiii 543 The Cystine Content of Hair and Other Epidermal Tissues
- 13 Buchthal H Ztschr f physiol Chem 1907 lvi 474 Über das Mengenverhältnis des Cystins in verschiedenen Hornsubstanzen
- 14 Laurens H Physiol Rev 1928 viii 1 The Physiological Effects of Radiation
- 15 Clark J H Physiol Rev 1922 ii 277 The Physiological Action of Light

- 16 Sullivan M A U S Pub Health Rep 1930 Suppl. 1 p No 86-1
Studies on the Biochemistry of Sulphur VII The Cystine Content of
Purified Proteins
- 17 John O and Malmros H J Biol Chem 1929 lxxxiii 115 An Im-
proved Form of Folin's Micro Method for Blood Sugar Determinations
- 18 Koch F C, and McMeekin T L J Am Chem Soc 1924 xlvi 2026
A New Direct Nesslerization Micro-Kjeldahl Method and a Modifi-
cation of the Nessler-Folin Reagent for Ammonia
- 19 Goldberger I and Linner, W F U S Pub Health Rep 1924 xxxix 87
A Study of the Treatment and Prevention of Pellagra
Ibid 1925 xl 54 A Study of the Pellagra-Preventive Action of Dried
Beans Casein Dried Milk and Brewer's Yeast with a Consideration of
the Essential Preventive Factors Involved
- 20 Goldberger I Wheeler G A, Lillie R D and Rogers J M U S
Pub Health Rep, 1926 xli 207 A Further Study of Butter, Fresh
Beer and Yeast as Pellagra Preventives
- 21 Mulder Cited by Hammarsten O and Hedlin, S Physiological Chem-
istry 7th Edition Translated by Mandel J A, John Wiley and Son
Inc New York 1914
- 22 Lungecker H Ztschr f physiol Chem 1921 cxv 38 Vergleichend
Untersuchungen über die chemische Zusammensetzung von men-
schlichen Nägeln aus verschiedenen Lebensaltern
- 23 Sullivan M A and Hess W C J Biol Chem 1932 xcvi Scientific
Proceedings p xxx Cystine Studies in Arthritis
- 24 Goldberger I and Linner, W F J A M A 1922 lxxix 2132 An
Amino-acid Deficiency as the Primary Etiological Factor in Pellagra
- 25 Smith J H Arch Int Med 1931 xliii 907 The Influence of Solar
Rays on Metabolism with Special Reference to Sulphur and to Pellagra
in Southern United States
- 26 Beidles I R Bramm W W and Mitchell H H J Biol Chem 1930
lxxxviii 623 The Relation between Cystine Deficiency in the Diet and
Growth of Hair in the White Rat
- 27 Sabry J, J Trop Med and Hyg 1931 xxxiv 303 On the Chemical
Nature of the 'Pellagra Toxin' and the Discovery of the Thiocyanate
Treatment of Pellagra

THE PRODUCTION OF EXPERIMENTAL PLAUT-VINCENT'S ANGINA IN THE DOG

BY HELENE WALLACE E. W. WALLACE AND O. H. ROBERTSON

*(From the Department of Medicine and the Department of Physiological Chemistry
and Pharmacology, the University of Chicago, Chicago)*

(Received for publication May 29 1933)

Since the original description of Plaut-Vincent's angina some fifty years ago a voluminous literature on this subject has accumulated. It is not the purpose of this paper to review the literature in any detail as this has been done elsewhere (1, 2). However it may be stated briefly that the disease was described in 1883 by Müller (18) in 1894 by Plaut (3) and in 1896 by Vincent (4) as manifested by sore and bleeding gums, foul breath and in severe cases by eventual ulceration of the mouth. They described the causative microorganisms as a spirochete and a fusiform bacillus. Since then these organisms have been found associated with pathological lesions in practically every part of the body but especially in the upper digestive system, the lungs and the genitalia.

Upon the isolation of the etiological agents the question was immediately raised as to whether they were separate entities or different forms of the same microorganism. Weaver and Tunchiff (5) succeeded early in cultivating the fusiform bacillus from a patient with Vincent's angina and on the basis of further work Tunchiff (6) has stated that she believes the spirochete and bacillus are the same organism in different stages of a definite life cycle. Some workers (7) have supported her but others (8) and in particular Pratt (9) have come to the conclusion that these are two distinct microorganisms.

The early literature stressed the infectious nature of the disease but more recent observations indicate that the causative organisms are found in the normal mouth rather constantly and that the inception of infection depends on the presence of certain predisposing factors. Pratt found fusiform bacilli in 100 per cent of the two hundred people routinely examined at a large hospital. She also found the fusiform bacillus constantly and the spirochete occasionally in normal monkeys, guinea pigs and rabbits. Other workers have reported analogous findings in these and other animals (2). Thus it would seem that the fusiform bacillus at least is present in the healthy mouth and is not in itself pathogenic. This is borne out by our work. Of forty dogs examined thirty-eight showed the bacillus and in those with poor teeth, spirochetes were usually present in the teeth pockets.¹

¹ Vincent's angina is a recognized disease of the dog's mouth.

Many attempts have been made to reproduce the disease experimentally. In 1923 Kline (10) produced abscesses in the traumatized gum of a guinea pig by injecting membranes from cases of Vincent's angina. Somewhat later Sauer (11) succeeded in producing similar abscesses in the untraumatized gingiva of a guinea pig by injection of material freshly obtained from human cases. He then injected into the lungs of rabbits pus from these experimental abscesses which showed spirochetes and fusiform bacilli in addition to other microorganisms. Of ten rabbits infected four developed lung abscesses, three gangrene, two pneumonia and one bronchiectasis. From certain of these lesions he isolated spirochetes and fusiform bacilli. Randall (12) injected scrapings from the gums of patients suffering from Vincent's angina into white rats and Werner (13) into kittens without success. Both workers used animals fed on a deficiency diet as well as normal ones. Wagener (13) traumatized the gums severely in some instances and made deep irritable pockets into which she injected the material but the wounds healed and no fusiform bacilli or spirochetes could be recovered. Lichtenberg, Werner and Luck (14) were unable to produce lesions in guinea pigs by the injection of pure cultures of fusiform bacilli into traumatized tissues but did find spirochetes and fusiform bacilli in the membranes which formed over ulcers induced by injury of the mouth in normal guinea pigs.

While the above data indicate that experimental lesions of the gum and lung have been induced by the implantation of spirochetes and fusiform bacilli the reproduction in animals of the clinical symptoms of Vincent's angina has not been successful.

In the spring of 1931 one of the authors (15) observed that a group of the dogs which he had been injecting daily with one of the squill glucosides, namely, scillaren B, developed a peculiar mouth condition similar to that found in Plaut-Vincent's disease. Smears from the mouths of the dogs showed the typical fusiform bacilli and spirochetes in large numbers. This observation suggested a possible method for the experimental production of Vincent's angina.

METHODS AND MATERIALS

mal was injected daily intravenously. The dogs injected with scillaren B were not run in a single series but one or two at a time simultaneously with other animals receiving different squill glucosides. A total of forty eight dogs were used. Each drug was tested on eight dogs. Conditions of care, hygiene and type of food were identical for all the dogs.

The dogs used in the present experiments were selected primarily for their good teeth and gums; they were all lively and in excellent condition. For the ease of handling the small terrier type was chosen. Temperature and pulse were taken twice daily, weight and (in the case of five dogs) a white count once a day. Detailed records by means of sketches and notes were made daily of the teeth, gums and mouth of each animal. At the same time smears were taken from each side of the mouth, the incisors and any suspicious pockets. The smears were stained by Tunchiff's method³. All the animals were kept under excellent conditions and were well fed. The food given contained adequate amounts of the various vitamins.

A saline solution of the drug was injected daily into the antebrachial vein of the dogs' foreleg. About 0.30 of the lethal dose was given for the first three days and subsequently 0.25 of the lethal dose until death. (The lethal dose as measured by the Hatcher Brody cat method is 0.1 mgm. per kilo of body weight.) Photographs were taken of the dogs months before the injection of the drug was begun in various stages of the disease and in two cases five minutes after death.

Autopsies were performed immediately after death. Particular attention was paid to the mouth, esophagus, stomach, trachea, lungs, heart and kidneys. Tissues from ulcerating areas as well as normal and only slightly affected regions were excised and sectioned. These tissues were usually fixed in Zenker's acetate (in the case of the silver methods for spirochetes 10 per cent neutral formalin was also used). The sections were for the most part stained with Giemsa's stain prepared by Grubler, as this was found to stain both the spirochetes and fusiform bacilli distinctly and also to give the best histological picture. Levaditi's silver method for spirochetes was also of value.

During the course of the disease in the animals numerous cultures from the mouth were made.

EXPERIMENTAL

In the present study eleven dogs, six males and five females, were injected with scillaren B. All of them developed the characteristic lesions and bacteriological findings of Vincent's angina. In three dogs (4, 5 and 8, Table I) the lesions were minimal, consisting of redness and sensitivity of the gums with some bleeding, discoloration of the teeth and the presence of numerous fusiform bacilli and some spirochetes. Two of these three animals, 5 and 8, both of them very young dogs, died of an acute respiratory infection at this stage.⁴ Dogs 1, 3, 10 and 11 exhibited a well marked clinical picture of the disease with one or two ulcerations. Dogs 2, 6, 7 and 9 developed a severe form of the infection. In all these eight

³ The stain made up of 90 cc. of a saturated 95 per cent alcoholic solution of Grubler's gentian violet added to 10 cc. of 5 per cent phenol was poured over the dried smears and steamed for five minutes. The slides were then washed with tap water, blotted with filter paper and examined under oil.

⁴ Not *fusiform* spirochetes as far as could be determined.

TABLE I
Summary of data

Dose number	Sex	Weight		Temperature				Protein		White blood cells	Number of days infected*	Severity of disease	Additional data
		Initial kg.	Final kg.	Rectal °C.	Rectal °F.	Body at death	Rectal at infection	Dry and fat of abomasum					
1	M	11.25	6.9	101.5	101.3	101	114	14h—60	10, 24, 14	7 D	7 D	Severe	Different between B Powder and More potent?
2	F	9	5.1	102.4	101.2	100	116	50h—48	7.5, 9, 3.5	11 D	11 D	Very severe	
3	M	7.1	5	102	101	99.6	110	120h—56	8, 12.5, 7	16 D	16 D	Severe	
4	M	14.4	11.1	102	101	101	100	280h—28	14, 16, 16	25	25	Mild	Drug discontinued. Animal recovered (See text)
5	M	11.1	5.9	101	100.4	98.6	116	100h—70	8, 20	12 D	12 D	Moderate	Died of respiratory infection
6	F	9.5	7.1	102	100	98.5	116	80h—52	—	15 D	15 D	Very severe	
7	F	10.1	9.2	102	101	105	124	60h—50	—	12 D	12 D	Severe	
8	M	10.1	6.5	101.8	101	99.5	110	3d—55	—	5 D	5 D	Moderate	Died of respiratory infection
9	M	10.1	6.5	101.8	101	99.5	110	Sub—45	—	15	15	Very severe	Animal allowed to recover. Injected 2d and 3d times. Subsequent at tacks less severe
10	F	10.1	10.1	101.2	100.8	100	120	90h—42	—	14 D	14 D	Severe	
11	F	12	8.1	101.8	100.6	100	116	70h—52	—	11 D	11 D	Severe	

* Days infected until death with the exception of No. 4 and No. 9
D = Died

animals the molars became black and the canines a very dark brown. Ulceration of various parts of the cheeks and tongue occurred. These ulcers were usually one to two centimeters in diameter. Several were considerably larger. There was much bleeding from the lesions.

As the disease progressed in a similar fashion in all the dogs a general description may be made. During the first two days the dogs were apparently normal but after the injection on the third day, they all vomited profusely and were somewhat depressed. By this time smears from the mouth usually showed a decided increase in the number of small, short fusiform bacilli. By the fourth or fifth day vomiting was acute and very little food was eaten. The teeth generally the posterior molars and canines were somewhat discolored the gum margins showed a narrow line of inflammation and the smears from these regions contained quantities of short fusiform bacilli and a sprinkling of spirochetes. By the sixth day the dogs were no longer lively or responsive although the vomiting was not as acute. The gums were markedly inflamed and bleeding, the teeth darker, and the smears showed larger and more typical cigar shaped bacilli and also spirochetes. From the seventh to the fourteenth day the teeth became progressively darker until all but the incisors were a dark brown the gums bled profusely and were very friable and ulcers formed on the gums cheek and tongue (Fig 1). The smears showed large typical fusiform bacilli and spirochetes. Quantities of the latter were found wherever there was a bleeding and necrotic pocket.

During the injections the dogs lost a great deal of weight in some cases almost half the original body weight. There was no fever but rather a drop in temperature during the last few days of the disease.

The white blood cell counts made on the first three dogs showed a decided increase at first but one or two days before death the leukocytes dropped considerably though not always below what might be considered normal. In Dog 4 the white count did not go down but remained at a rather high level. This animal exhibited the mildest lesions in the series. Dog 5 which died of a concurrent respiratory infection showed a rise in the white blood cells to 20 000. However the leukocytosis apparently did not hinder the development of the Vincent's angina.

The animals died on an average about the fourteenth day in ventricular fibrillation. However one dog died as early as the seventh day and another (Dog 4) did not succumb to twenty eight injections. Most of the dogs were injected until they succumbed from the cumulative effects of the drug. In two animals the drug was discontinued as shown in Table I (Dogs 4 and 9). Dog 9 recovered from a severe attack of the experimental disease and two months later when a smear from the mouth showed only the few fusiform bacilli usually found in normal dogs he was again injected and developed a pronounced Vincent's angina as before. After a second recovery and a three months interval a third attack was induced. The second and third attacks were not as severe as the first.

used for other experimental purposes.⁵ Dogs 5 and 8, the two animals that died of a respiratory infection had a final temperature of 104 and 105° F. respectively. However, early in the course of the disease their temperatures were normal.

As shown in the table the average pulse rate for three days before injection was about 112. After the injection of the drug an initial progressive slowing of the heart occurred. Electrocardiograms taken at the time when the rate was slowest showed the heart to be in a condition of partial to complete block. Apparently the drug in this phase of its action progressively lengthened the conduction time until there was complete dissociation between auricular and ventricular contractions. The day on which the pulse was slowest generally occurred about the middle of the injection period. Later as more toxic amounts of the drug accumulated the heart rate increased. Apparently the ventricular musculature itself was stimulated. Ectopic foci appeared at various points in the ventricle, producing irregular QRS-T complexes of high potential and bizarre shapes. Before death the heart rate was usually too rapid to be counted.

Attempted transmission

Several control animals were kept in the room with the injected dogs and although they were allowed to eat out of the same pan as dogs with well advanced cases of Vincent's angina they did not contract the disease. Swabs were then taken from the necrotic pockets in the gums of Dogs 6 and 7 and pushed into the gum margins of the control animals without any apparent effect save that in one dog, whose physical condition was somewhat poorer than the others, there was some transient local redness and tenderness of the gums.

Pathology

At autopsy lesions were found only in the mouth. All the internal organs appeared normal. An extremely foul odor came from the mouth when it was opened. The teeth were badly discolored, the canines and molars being the most affected. Necrotic pockets filled with spirochetes were usually present at the base of these discolored teeth. In only one animal (Dog 3) were the incisors affected. In this instance the molars were less involved than the incisors and the single ulcer present was at the base of the lower incisors. In Dogs 2, 6, 7 and 9 there were extensive cheek ulcers on both sides of the mouth. The first three of these dogs also had from one to five ulcers on the tongue, one to two centimeters long and one half to one centimeter wide. The largest ulcers were those on the cheek of Dog 7 where the whole right side of the mouth was involved. All the ulcers, although irregular in shape, had well defined margins and

⁵ This dog was subsequently found to be unusually resistant to infection with pneumococci and streptococci.

fusiform bacilli only became numerous about the sixth day at which time spirochetes and necrosis also appeared. The spirochetes were found in abundance in the pockets and necrotic regions (Fig 2). The typical

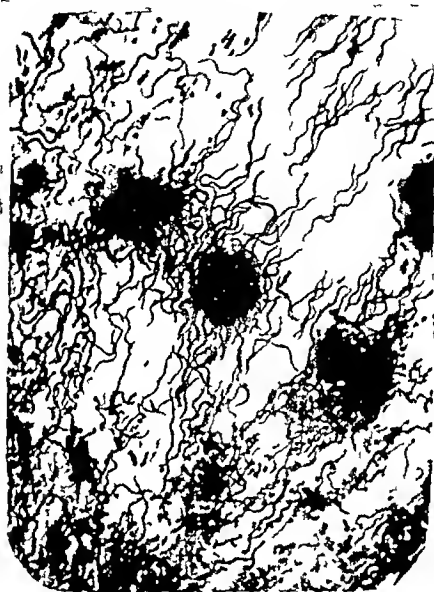


FIG 2 Dog No 9

Smear taken from necrotic pocket in gum on tenth day of disease shows masses of spirochetes 1200 X

Vincent's angina smear (Fig 3) could be obtained at the end of the first week of injection and at any subsequent time. In this microphotograph the spore like character of the fusiform bacilli may be seen. Long filaments of fusiform bacilli were not uncommonly observed (Fig 4). In some cases they resembled the filamentous forms seen in the cultures and sections. The smears at this time showed practically no other organisms than the fusiform bacilli and spirochetes.

A study of a number of human cases of Vincent's angina gave analogous findings. They showed all the variations of the fusiform bacilli that were present in the dogs' mouths. Likewise in the human cases the spirochetes were found in the teeth pockets or deep in the necrotic tissue such as occurred in tonsillar crypts.

Fig. 3. Dog No. 6
Smeear made from gum on ninth day of disease shows typical sporotrich
and histiocyte reaction of *Amecina magna* (120x)

Cultures were taken from the dogs' mouths when the smears showed many spirochetes and fusiform bacilli. A number of cultures were also made from the pockets about the teeth, ulcers, and regions where the smears showed a predominance of one microorganism or the other. Most of the cultures were made with material from Dog 9.

Various culture media were used. The fusiform bacilli grew fairly well on rabbit's blood agar (one part of the blood to three parts of beef infusion agar, pH 7.4 to 7.6) but not nearly as readily as on ascitic agar (one part of ascitic fluid to three parts of a beef infusion agar, pH 7.4 to 7.6). Ascitic agar and a beef infusion—ascitic broth (three parts of one per cent dextrose beef infusion broth to one part ascitic fluid, pH 7.4 to 7.6) were our most satisfactory media. With Loeffler's medium recommended by Tunchiff, we were less successful. We also used Noguchi's leptospira medium (17) in an attempt to grow the spirochetes.

All the cultures were grown anaerobically. Although we tried anaerobic plates as well as anaerobic jars in which the air had been evacuated and replaced with CO₂ or nitrogen, we found that Wright's anaerobic method besides being the simplest was also the best for our purpose. Test tubes were stoppered by cotton saturated with a five per cent NaOH solution. A small amount of pyrogallol acid was placed on top of this and the tube corked and sealed with paraffin. Incubating temperature was 37° C.

We were able to isolate colonies of the fusiform bacilli quite readily after forty-eight hours incubation. They were minute and while not absolutely transparent, they seemed so when contrasted with the opaque colonies of cocci which formed the bulk of the growth. Single colonies were transferred to ascitic agar slants and were maintained in pure culture without difficulty. Transplants to fresh media were made every three days for two weeks and subsequently every two weeks for three months. After some months the bacillary forms became more or less degenerate although a slant examined eleven months subsequent to the initial culture still contained some recognizable fusiform bacilli. At no time were we able to cultivate the spirochetes—even when the inoculum was obtained from pockets showing practically a pure growth of spirochetes—nor did we find spirochetes at any time in our cultures of fusiform bacilli. Subcultures of fusiform bacilli in leptospira medium yielded only fusiform bacilli.

The fusiform bacilli in the pure cultures were small at first but grew rapidly to the usual size and subsequently into the long filamentous forms which at times stretched completely across the microscopic field. They were also often wavy and to the casual observer might be taken for spirochetes. They were however much larger than the Vincent's spirochete and under dark field examination were seen to be non motile.

As a rule once the fusiform bacilli had attained the filamentous stage they showed no further change. Occasionally small fusiform bacilli appeared in cultures in which only filamentous forms had been observed for

that the injection of scillaren B disturbs metabolic processes in such a manner as to lower the resistance of the buccal mucous membrane to the spirochetes and fusiform bacilli normally present. Whether this deleterious effect of the drug is principally a generalized or a local one we do not know. While it is true that the animals all showed marked gastro intestinal disturbances and an associated loss in weight these same effects were produced to an equal degree by the other squill derivatives employed but without the subsequent development of fuso spirochetal mouth lesions except in one instance. Thus it would seem that either the general effect of scillaren B is of a peculiar nature or that it produces a characteristic local disturbance not shown by the five other closely related compounds. The excretion of the drug locally in the mouth cannot be ruled out as a cause of the lesion but neither can it be determined as such since the amounts injected are far too small to render recovery of the drug possible. It is of course conceivable that the production of the lesions in the mouth by this one squill glucoside is due to impurities in the preparation rather than to the compound itself. Of two lots of scillaren B used one was found to cause the development of the experimental disease much more quickly than the other.

The data presented indicate that a leukopenia is not a factor in favoring inception of the disease nor does a leukocytosis appear to influence its course.

Our unsuccessful attempts to transmit the disease to healthy dogs while too few in number to permit conclusions suggest that the fusiform bacilli and spirochetes may not be pathogenic for the normal animal even when applied to the gums and mucous membranes of the mouth in large numbers. In a recent paper, Lichtenberg and his co workers (14) question the pathogenicity of these micro organisms for normal human beings. However there are well authenticated reports in the literature of small epidemics of Vincent's angina among normal persons living under apparently healthful conditions. This leads one to infer that either the normal dog possesses a greater natural resistance to the fuso spirochetal micro organisms than do human beings or that the strain found commonly in the dog differs in pathogenicity from that occurring in the human mouth.

With the evidence available we feel that it is not yet possible to draw definite conclusions concerning the identity or non identity of the spirochete and fusiform bacillus. Our cultural studies do suggest that these are two distinct microbial forms. If the fusiform bacillus and the spirochete represented different phases of a single life cycle one might expect that in a culture medium highly favorable to the multiplication of the bacillary form the spirochetal phase would develop. However since we have not been able to contrive sufficiently favorable conditions in the test tube for the growth of spirochetes freshly isolated from the lesions we have no means of knowing whether or not spirochetes would evolve from fusiform bacilli were a suitable growth medium provided.

- 9 Pratt, J S, J Infect Dis, 1927, xli, 461 On the Biology of B Fusiformis
- 10 Khne B S J Infect Dis, 1923, xxxii, 481 Experimental Gangrene
- 11 Smith D T J Infect Dis 1930, xlv, 303 Fusospirochetal Disease of the Lungs Produced by Cultures from Vincent's Angina
- 12 Randall, S J J Dent Research, 1931, xi, 49 An Attempt to Produce Vincent's Infection in the White Rat
- 13 Wagener, E H et al, J Dent. Research, 1930, x, 591 The Laboratory Diagnosis of Vincent's Infection
- 14 Lichtenberg H H, Werner M and Lueck E V, J A M A, 1933, c, 707 The Pathogenicity of the Fusiform Bacillus and Spirillum of Plaut-Vincent.
- 15 Wallace E W and Van Dyke, H B, pending pub J Pharmacol and Therap, 1933 Cumulative Poisoning by Squill Derivatives and by Ouabain
- 16 Kolle, W, Kraus, R. and Uhlenhuth P Handb d Path Mikro organismen 1928, Volume V, Part I 607 "Bacillus fusiformis" (By H A Gins)
- 17 Noguchi, H, 'The Spirochaetes' Newer Knowledge of Bacteriology and Immunology University of Chicago Press, 1928, p 452
- 18 Miller, W D N England J Dent 1883, ii, 229 Further Contributions on the Subject of Dental Caries (Quoted by Harrell (1))

THE EFFECTS OF ALTERNATE SUCTION AND PRESSURE ON BLOOD FLOW TO THE LOWER EXTREMITIES

By EUGENE M. LANDIS AND JOHN H. GIBBON, JR.

*(From the Robinette Foundation of the Hospital of the University of
Pennsylvania Philadelphia)*

From the therapeutic standpoint patients with peripheral vascular disease are divided into two groups, (a) those whose symptoms are due to simple spasm with no, or very slight, organic vascular obstruction and (b) those whose symptoms are due primarily to advanced organic disease of the arteries. The symptoms of the first group can usually be alleviated by producing vasodilatation with drugs, diathermy, local heat in the form of the warm cradle, or sympathetic ganglionectomy. The efficacy of these therapeutic procedures depends upon local dilatation of the peripheral blood vessels.

In more advanced stages, however, obliterative structural disease has usually progressed until the arterial vessels are transformed into rigid tubes. These diseased vessels have not only abnormally small lumina but are also more or less incapable of dilatation even when vasomotor tone is reduced by nerve block or by sympathetic ganglionectomy. When the arteries have thus lost their power of dilating the therapeutic procedures mentioned above fail to increase blood flow and therefore fail to improve the nutrition of the tissues. Eventually many of these patients with advanced organic disease of the arteries suffer from trophic changes, ulceration and gangrene leading finally to amputation. Any procedure which tends to increase blood flow in the presence of organic occlusion might be of benefit in delaying or possibly in preventing the appearance of gangrene.

According to Poiseuille's law the amount of fluid flowing through a rigid tube depends upon the fall in pressure along the tube. If other factors remain constant doubling the peripheral fall in blood pressure should approximately double the amount of blood flowing through the rigid arterial vessels. The total fall in pressure in the peripheral vascular system may be increased in two ways: (a) by elevating systemic blood pressure or (b) by diminishing capillary and venous blood pressures. The first method is impracticable for numerous reasons. It seemed possible, however, that capillary and venous blood pressures might be reduced temporarily to negative values (i.e., below atmospheric pressure) by applying suction to the skin of the extremity.

The full effect of externally applied negative pressure can only be obtained if there be a reservoir capable of accommodating the blood which

has flowed through the narrowed arteries. The capillaries and veins which are not constricted offer such a reservoir—or limited capacity. Landis and Gibbon (1) observed that inflating a pneumatic cuff on the upper arm to a pressure of 30 cm. water for 5 minutes increased the volume of a segment of forearm by 25 cc. of which 3 cc. consisted of extravascular fluid while 22 cc. consisted of blood trapped in the congested peripheral vessels. Similarly, a venous pressure of 60 cm. water during 6 minutes increased the volume by 38 cc. of which 7 cc. was extravascular fluid and 31 cc. was blood in the congested vessels. The total volume of the segment of forearm was 720 cc. so that at pressures of 30 and 60 cm. water the reservoir available was 3 and 4 cc. per 100 cc. of arm respectively. Average blood flow in the normal forearm amounts usually to less than 5 cc. per minute per 100 cc. of tissue (Lewis and Grant (2)). Hence, with a slight rise of pressure the veins and capillaries can accommodate the blood flowing through normal vessels during a period of approximately one minute.

From these observations it seemed possible that the periodic application of negative pressure to the surface of the lower extremity might be expected to increase blood flow even though the arterioles had been eliminated into rigid tubes by reason of structural disease. Moreover, if the suction were applied for relatively short periods of time the efficiency of the method would be greater since the available reservoir would not be filled to capacity during any one suction period. The accumulation of blood in the capillaries and veins must lead to stretching of their walls, the resistance to further distention increasing as the amount of trapped blood becomes greater. This resistance would of course, lessen the effect of external suction on the peripheral drop in blood pressure particularly if suction were prolonged. It seemed essential, therefore, not only to use relatively brief periods of suction but also to empty the capillary and venous beds of their contained blood after each brief suction period, so that space might be available for the accommodation of fresh arterial blood to be drawn in during the succeeding suction period. Therefore, suction and pressure were applied alternately.

The effects of alternate suction and pressure on flow have been studied in a circulatory schema and in the lower extremities of normal subjects and of patients with peripheral vascular disease. This paper provides objective evidence that blood flow can be increased by this means. The clinical value of this procedure will be considered in later publications. It seemed advisable first to demonstrate objectively and under controlled conditions, the correctness of the working hypothesis.

APPARATUS AND METHOD

was 28 cm wide 75 cm long 28 cm high at the proximal end and 34 cm high at the distal end. These dimensions permitted the lower extremity to be inserted to a point approximately 8 inches above the knee joint (Fig 1). The upper

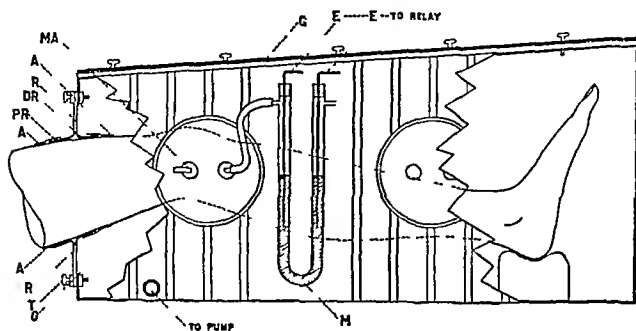


FIG 1 DIAGRAM OF ALUMINIUM BOX USED TO APPLY SUCTION AND PRESSURE TO THE LOWER EXTREMITY

For description see text

surface of the box could be removed and consisted of a sheet of shatter proof glass (G) mounted in a holder which was screwed down upon a rubber gasket. The foot and leg could thus be observed during the application of suction and pressure. The proximal end of the box contained a circular opening (O) measuring 25 cm in diameter which was fitted with a rubber cuff screwed tightly against the end of the box by means of thumb screws (T). The cuff consisted of a semi rigid ring (R) of rubber and canvas with an external diameter of 28 cm and an internal diameter of 14 cm. The internal portion of the cuff consisted of two leaves of lighter flexible rubber having a thickness of 1.5 to 2 mm. These leaves were conical in shape, the smaller end of one (PR) being directed toward the hip while the smaller end of the other (DR) was directed toward the knee. The dimensions of the cuff were adjusted for different subjects so that the flexible rubber fitted snugly against the skin of the thigh. The fit was made as accurate as possible in order to avoid both excessive venous congestion and excessive leakage of air. The rubber was held lightly in contact with the skin by adhesive tape (A). The heel rested on a sand bag and for greater comfort the lower thigh was supported by sand bags placed just within the rubber diaphragm.

The interior of the box communicated through three-quarter inch rubber tubing with an air pump of the Crowell type (size 2A) having a capacity of 13 liters per minute at atmospheric pressure. The interior of the box also communicated with a mercury manometer (M) made of glass tubing with an internal diameter of 1 cm. A metal electrode (E) was inserted into each limb of the manometer. These electrodes were connected with a difference of potential and with the coil of an electrical relay. The contacts of the relay operated a one half horse power A C (Westinghouse Type RV) motor connected by a leather belt to the air pump. A motor driven 3 way metal valve placed the box in communication with the intake pipe of the pump for 25 seconds, and with the

output of the pump for 5 seconds. The manometer and valve therefore controlled automatically (by the position of the two electrodes in the manometer) the amount of suction or pressure applied, while the valve controlled the duration of the suction and pressure periods.

In all but two of the observations here reported a negative pressure of 120 mm Hg was applied for periods of 25 seconds, alternating with a positive pressure of 80 to 100 mm Hg for periods of 5 seconds. It required approximately 3 to 5 seconds to change the pressure within the box from -120 to $+120$ mm Hg. For observing the final pressures more accurately a second manometer communicated with the interior of the box through a separate opening (*M4*).

The air expelled by the pump warmed in the process of compression. Since changes in blood flow were detected by recording skin temperature it was necessary to cool the air entering the box during the pressure periods. The air was therefore passed through a honeycomb radiator immersed in water at 6 to 10°C . This prevented excessive warming of the air surrounding the lower extremity.

Changes in cutaneous blood flow in the lower extremity were measured qualitatively by observing skin temperature. Three copper-constantan junctions passed into the interior of the box through a rubber stopper inserted in the side wall. One junction was fixed to the skin at the base of the nail of the first toe, another junction was similarly situated on the skin of the third toe. Each junction was covered with one layer of adhesive plaster. The third thermal junction was suspended near the foot to record the temperature of the air around the extremity.

Since in these observations increase in blood flow was identified by elevation of skin temperature it was essential to control the observations very carefully. Therefore, while one lower extremity was placed in the aluminum box the other lower extremity was used as a control and inserted into a second box having the same dimensions and the same general shape as the aluminum box. This control box was fitted with one 10-watt and one 50 watt lamp, which could be turned on separately or together in order to keep the temperature of the air in the control box always above that of the air in the aluminum box.

Conditions were arranged to favor greater cooling of the extremity exposed to suction and pressure. First, the air temperature in the control box was higher than that in the aluminum box. Second, the air in the control box was still while that in the aluminum box was moving due to alternate removal and introduction of air during suction and pressure respectively. Third, the extremity in the aluminum box was slightly congested by the rubber cuff while the extremity in the control box was not congested; the space between the skin of the thigh and the proximal end of the control box being closed loosely with cotton. The conditions were thus arranged to favor cooling of the experimental extremity as compared to the control extremity, so that if the extremity exposed to suction and pressure became warmer than the control extremity, the change might be safely ascribed to increased blood flow.

OBSERVATIONS

fluid through a partially obstructed rigid tube. In the schema various lengths of capillary glass tube simulated an arterial obstruction while a rubber bag simulated the distensible capillary and venous beds. A valve was inserted in the outflow (or venous) tube leading from the bag to simulate the venous valves.

The rate at which water flowed through the system under a pressure of 80 mm Hg (109 cm water) was first measured over several periods of six minutes without pressure variations, i.e., with atmospheric pressure both inside and outside the box. The first column in Table 1 shows the single and average measurements of the outflow in cubic centimeters per minute. Suction (-120 mm Hg) was then repeatedly applied to the portions of the schema within the box for varying periods—25, 55, 85, and 115 seconds—with alternate periods of pressure.

Flow was uniformly greater during suction and pressure. The percentage increase in flow is recorded in the right half of Table 1. Suction for 25 seconds and pressure for 5 seconds increased the outflow by 45 to 63 per cent. With longer periods of suction slightly greater increments in flow were observed since the total drop in pressure was increased for a slightly greater proportion of time, relatively less time being wasted in changing from negative to positive pressure.

B. Observations on normal subjects

1. The effects of suction and pressure on the rate of cooling of the previously warm lower extremity

At low room temperatures the extremities of normal subjects cool rapidly as a result of peripheral vasoconstriction which serves to conserve heat by diminishing radiation. As shown by Gibbon and Landis (3) when the forearms are immersed in warm water maximal vasodilatation is produced in the lower extremities, and the surface temperature of the digits rises to 32° C or more. In this series of observations the subjects were exposed to low room temperatures and maximal vasodilatation was first produced in the lower extremities by immersing the forearms in warm water. The rate at which the lower extremities cooled following such maximal vasodilatation was observed both with and without pressure changes in order to ascertain whether alternate suction and pressure modified the rate of cooling.

The subjects were seated on an examining table with the legs extended horizontally while the back rested against a support at an angle of 45 to 60° . The right lower extremity was placed in the control box, the air in which was kept slightly warmer than that of the aluminum box. The left lower extremity was inserted into the aluminum box through the rubber cuff.

Figure 2A shows the characteristic result obtained when the two extremities were first warmed through peripheral vasodilatation and then

TABLE I
Effects of alternate suction and pressure on the flow of water through a circulation schema

Outflow from venous end of schema		With suction and pressure				Percentage increase in outflow produced by suction and pressure			
Without suction and pressure cc. per minute	Suction 25 seconds Pressure 5 seconds cc. per minute	Suction 55 seconds Pressure 5 seconds cc. per minute	Suction 85 seconds Pressure 5 seconds cc. per minute	Suction 115 seconds Pressure 5 seconds cc. per minute	Suction 25 seconds Pressure 5 seconds per cent	Suction 55 seconds Pressure 5 seconds per cent	Suction 85 seconds Pressure 5 seconds per cent	Suction 115 seconds Pressure 5 seconds per cent	
150	217	217	211	211.5	15	63	61	61	
152 115 112 110 111 110	213 212 212	212.5	252 252	251.5	63	70	76	78	
50 49 48 47		67 76 75 77	75 75 79 79			58	61		
47.5 47.5 47.5	71 71 71	76 79 79	77 79 79		58	60	62		

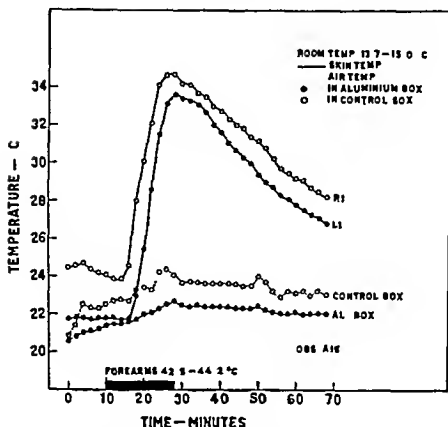


FIG 2A CONTROL OBSERVATION SHOWING COOLING OF THE DIGITS OF THE LOWER EXTREMITIES WITHOUT SUCTION AND PRESSURE

In this and subsequent charts R1 indicates the skin temperature of the right first digit, L1 the skin temperature of the left first digit

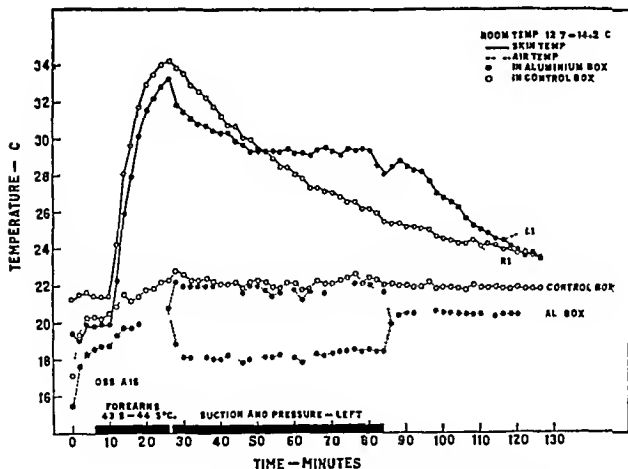


FIG 2B SHOWING COOLING OF THE DIGITS DURING APPLICATION OF SUCTION AND PRESSURE TO THE LEFT LOWER EXTREMITY

allowed to cool in their respective boxes without stimulation and pressure being applied to the left lower extremity. As was to be expected, on account of the warmer air in the control box, the temperature reached during vasodilatation was higher in the right extremity. After the forearm was removed from the warm bath, vasoconstrictor tone increased and the right and left extremities cooled at approximately the same rate, the right extremity remaining throughout slightly warmer than the left. The subjects were chilly or shivering while the lower extremities cooled.

Six such control observations are summarized in Table 2. Room temperature ranged from 10.7 to 16.3° C. At the beginning of the cooling period the surface temperatures of the digits were all above 33° C., the right digits being warmer than the left. The duration of the cooling period varied from 38 to 58 minutes and in this time the temperatures of all the digits became lower. With one minor exception the left digits remained as cool as, or cooler than, the corresponding right digits throughout the cooling period. The temperatures of the air in the aluminum and control boxes were read every two minutes. The figures recorded in Table 2 as "average temperature of air in boxes" at the beginning and end of the cooling periods represent respectively the average of the five readings made during the first 10 minutes and the last 10 minutes of the cooling period. It may be observed that the air in the control box was warmer than the air in the aluminum box by 1.1 to 1.7° C.

The relation between the surface temperatures of the right and left digits is summarized in the last two columns of Table 2. The temperature of the right first digit at the beginning of the cooling period has been subtracted from that of the left first digit observed at the same time. The left and right third digits have been similarly compared as regards temperature. The minus sign indicates that the left digit was the cooler by the amount given. At the beginning of the cooling period the left digits were from 0.1 to 2.5° C. cooler than the right. At the end of the cooling period the same relation existed in exaggerated form, the left digits being, with one exception, from 0.0 to 3.8° C. cooler than the right. The single exception occurred in Observation 116 in which the left third digit became 0.3° C. warmer than the right third digit. The figures given in the lower right hand corner of Table 2 indicate that the left digits averaged 0.8° C. cooler than the right at the beginning of the cooling period and 1.8° C. cooler at the end of the period. These control observations show that under the environmental conditions mentioned the left digits remained definitely cooler than the right, i.e., a section and pressure were not applied to the left extremity.

TABLE 2
Cooling of feet without suction and pressure

Observation	Subject	Room temperature	Digit	Skin temperature		Duration of cooling period	Average temperature of air in boxes		Difference in skin temperature { L1 R1 L3 R3		
				At beginning of cooling period	At end of cooling period		Beginning of cooling period	End of cooling period	Beginning of cooling period	End of cooling period	
A16	G	C 13.7-15.0		C	C	minutes 40	C	C	C	C	
			L1	33.6	26.8						
			L3	33.1	25.8		22.5	22.0	-1.1	-1.4	
			R1	34.7	28.2				-0.5	+0.3	
			R3	33.6	25.5	23.9	23.1				
A26	G	11.0-13.8		L1	33.4	28.6	50				
			L3	33.4	26.0	22.9		22.3	-2.5	-2.9	
			R1	35.9	31.5				-1.2	0.0	
			R3	34.6	26.0	24.5		23.7			
A28	G	13.0-13.5		L1	34.0	25.3	38				
			L3	33.3	23.6	20.9		20.1	-1.0	-1.6	
			R1	35.0	26.9				-0.2	-0.4	
			R3	33.5	24.0	22.5		21.6			
A29	L	14.0-15.2		L1	35.9	32.9	50				
			L3	34.6	30.5	23.6		22.4	-0.2	-1.1	
			R1	36.1	34.0				-1.2	-2.1	
			R3	35.8	32.6	25.3		24.0			
A30	L	15.5-16.3		L1	34.7	29.3	58				
			L3	33.8	28.0	21.9		21.6	-0.1	-2.5	
			R1	34.8	31.8				-0.3	-2.4	
			R3	34.1	30.4	23.5		23.1			
A31	L	10.7-13.0		L1	33.4	27.3	56				
			L3	33.7	26.9	20.5		19.8	-0.3	-3.8	
			R1	33.7	31.1				-0.9	-3.6	
			R3	34.6	30.5	22.1		21.5			
							Average		-0.8	-1.8	

The air in the aluminium box became warmer during compression and cooler during rarefaction as was to be expected from physical principles. The range of these fluctuations during suction and pressure has been shown in Figure 2B (et seq.) by dividing the line showing air temperature in the aluminium box into two lines, of which the upper represents the

allowed to cool in their respective boxes without suction and pressure being applied to the left lower extremity. As was to be expected, on account of the warmer air in the control box, the temperature reached during vasodilatation was higher in the right extremity. After the forearms were removed from the warm baths vasoconstrictor tone increased and the right and left extremities cooled at approximately the same rate, the right extremity remaining throughout slightly warmer than the left. The subjects were chilly or shivering while the lower extremities cooled.

TABLE 3—(Continued)

Observation number	Subject	Room temperature	Digit	Skin temperature			Duration of suction and pressure (left only)	Duration of period without pressure changes	Average temperature of air in boxes			Difference in skin temperature { L1 R1 L3 R3			
				Beginning of period of suction and pressure	End of period of suction and pressure	End of period without pressure changes			Begin ning of pressure changes	End of pressure changes	End of period without pressure changes	Begin ning of pressure changes	End of pressure changes	End of period without pressure changes	
A19	L	C 150-158	L1 L3	C 33.6 34.0	C 31.2 29.4	C 27.8 27.5	minutes 59	minutes 36	C 21.5	C 21.9	C 22.4	C -1.3 -0.5	C +3.2 +1.9	C +0.4 +0.5	
				34.9 34.5	28.0 27.5	27.4 27.0	None	23.3	23.6	23.3					
				34.2 32.2	30.5 25.9	26.8 24.7	50	21.9	22.7	21.8	-0.1 -1.6	+1.8 -0.3	+0.3 +0.2		
A22	G	12.8-13.2	R1 R3	34.3 33.8	28.7 26.2	26.5 24.5	None	30	24.1	24.8	24.9				
				33.6 31.6	32.9 29.3	28.9 26.1	66	17.6	18.7	19.5	-0.6 +0.1	+2.6 +1.4	-1.1 -0.8		
				34.2 31.5	30.3 27.9	30.0 26.9	None	20.2	21.8	21.4					
A6*	L	14.2-15.8	R1 R3					46							
									Average			-0.8	+2.2	+0.4	

* Both feet spontaneously warm—arm baths not used.

maximum temperature observed during the pressure period and the lower represents the minimum temperature observed during the period of suction. The thermal junction recording the temperature of the air in the aluminum box was connected with the galvanometer during a complete suction and pressure cycle and the highest and lowest temperatures only were recorded. The galvanometer was of the low resistance type (Leeds and Northrup Type R) and was slightly overdamped. When relatively constant temperatures were being measured it required about 1 second for the galvanometer to come to rest. Positive pressure was applied for 5 seconds, a period slightly longer than that required for the galvanometer to reach equilibrium. The fluctuations in air temperature proved to be relatively uniform as shown in Figures 2B, 4-4, etc.

The air in the aluminum box was at the higher temperature for less than $\frac{1}{4}$ of the time while the lower temperature prevailed for over $\frac{3}{4}$ of the time. The average temperature of the air in the aluminum box would therefore be represented by a line slightly lower than midway between the maximum and minimum temperatures shown. Nevertheless, the air in the control box was kept warmer than the highest temperature recorded in the aluminum box since the effects of suction and pressure were being measured in terms of the cooling of the left, as compared to the right, extremity.

Under these conditions, suction and pressure modified the skin temperature of the left extremity as shown in Figure 2B. The right digits cooled uniformly and evenly during the entire period. The left digits, however, cooled more slowly during the application of suction and pressure. In the observation illustrated in Figure 2B during the early part of the suction and pressure period the left first digit was approximately 2° cooler than the right first digit. At the end of the suction and pressure period, 57 minutes later, the left first digit was approximately 3° warmer than the right. When suction and pressure were discontinued the left extremity began to cool much more rapidly and finally reached the same temperature as the right.

mum and the first five minimum readings. This figure is probably higher than true mean air temperature for reasons mentioned above. The air in the control box was kept approximately 2°C higher than this computed average and slightly higher than the maximum temperature in the aluminium box.

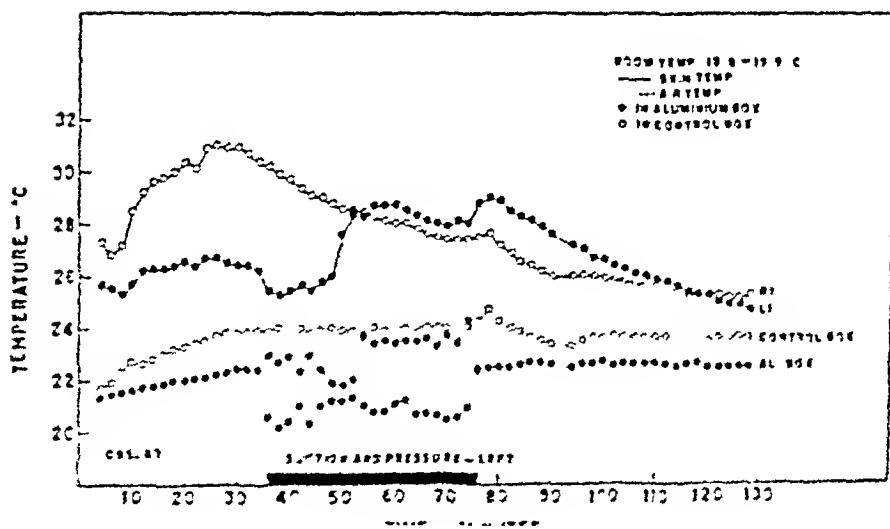
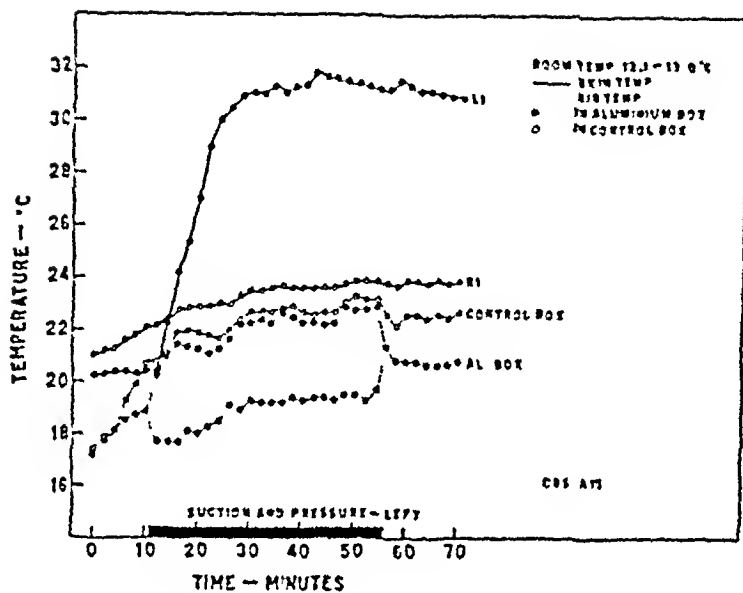
The relation between the surface temperatures of the right and left digits is summarized in the last three columns of Table 3. The temperature of the right first digit has been subtracted from that of the left first digit (*a*) at the beginning of pressure changes, (*b*) at the end of pressure changes, and (*c*) at the end of the period without pressure changes. The third toes have been similarly compared as regards temperature.

As in Table 2 a minus sign indicates that the left digit was cooler by the amount given while a plus sign indicates that the left digit was warmer. At the beginning of suction and pressure the left digits were, with one exception, from 0 to 1.7°C cooler than the right digits. At the end of the suction and pressure periods the left digits were, with one exception, from 0.2 to 4.5°C warmer than the right. In the two exceptions noted the left digit cooled much less rapidly than the right, though the difference was not enough to change the sign. During the subsequent period when cooling continued without the application of suction and pressure to the left extremity, the difference between the temperatures of the two extremities diminished conspicuously. In some instances the left extremity became once more the cooler, in every instance the difference in temperature became distinctly less.

The results are summarized in the average figures recorded in the lower right hand corner of Table 3. At the beginning of suction and pressure the left digits averaged 0.8°C cooler than the right. At the end of the suction and pressure period the left digits averaged 2.2°C warmer than the right. After a period without suction and pressure this difference diminished conspicuously, finally amounting to only 0.4°C . This forms a marked contrast to the control observations recorded in Table 2 in which without suction and pressure the left digits, instead of becoming warmer, cooled slightly more rapidly than the right or control digits. The difference in the rate of cooling must be ascribed to the relatively greater flow of arterial blood into the skin of the extremity exposed to suction and pressure.

2 The influence of suction and pressure on blood flow in the cool extremities of normal subjects

The effects on skin temperature of applying suction and pressure to the cool extremity were observed in order to ascertain whether blood flow could be increased in the presence of more or less marked vasoconstrictor tone. Room temperature was low, varying from 8.9 to 19.9°C . The subjects in these observations were usually cool or cold to the point of shivering. As was to be expected under these conditions skin temperatures



10 observations. With the beginning of suction and pressure the skin temperature of the left digits began to rise more or less rapidly, while the right digits either cooled or showed no rise in temperature other than might have been expected from the gradual warming of the control box.

As shown in the upper part of Table 4 suction and pressure increased blood flow in the cold extremity in 6 of 10 observations on two normal subjects. The left digits warmed by as much as 11.3°C while the right digits either cooled or warmed by not more than 2.2°C . Expressed for convenience in the form of average figures the results may be summarized by stating that in these 6 observations the left digits, exposed to suction and pressure, became warmer by 6.3°C while the right digits, though exposed to a higher air temperature, became warmer by only 0.3°C .

The lower section of Table 4 and the first portion of Figures 4A and 4B summarize the observations in which suction and pressure alone did not influence blood flow conspicuously. The data in Table 4 apply only to the period when suction and pressure were used without immersion of the forearms in warm water, the latter being considered in the next section. These four observations may be summarized by stating that the left digits exposed to suction and pressure (before diminishing vasoconstrictor tone by immersing one forearm in warm water) showed, on the average, no change in temperature while the right digits became cooler by 1°C though the latter were exposed to a warmer air temperature. This difference is doubtless too small to be of any real significance, though in qualitative agreement with the other observations in which larger changes were observed. High vasoconstrictor tone can apparently diminish the effects which suction and pressure exert on blood flow.

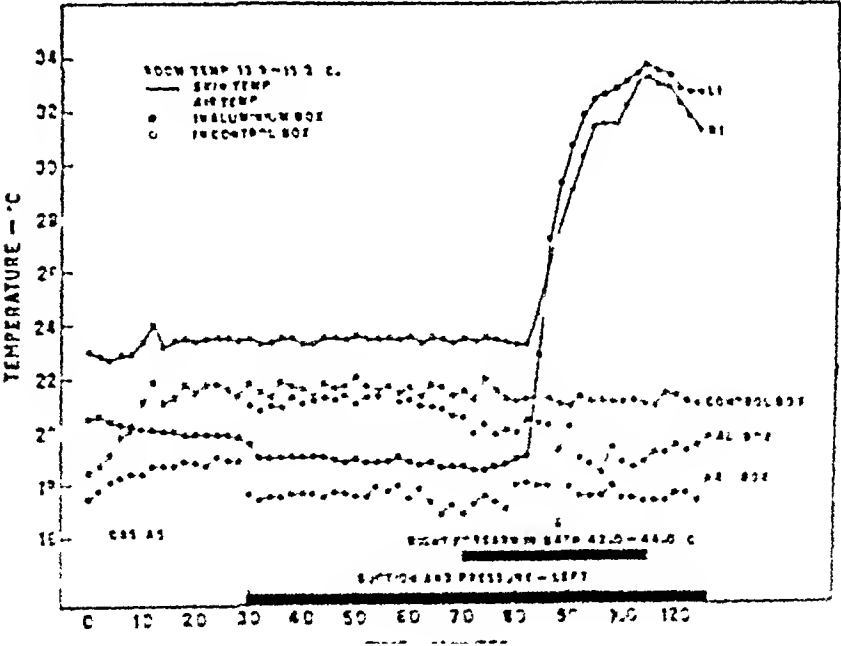
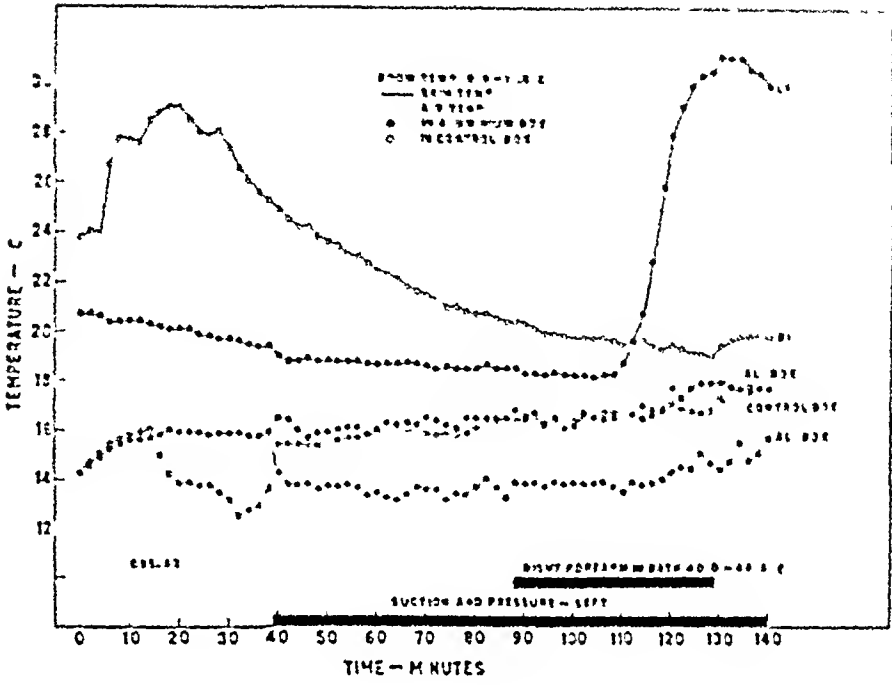
It was mentioned previously that theoretically the effects of suction and pressure would be expected to depend upon the presence of an adequate reservoir to accommodate the blood drawn through the arterioles during the suction period. It has been shown by Lewis (5) that during vasoconstriction the minute vessels of the skin can resist internal positive (or external negative) pressures of 50 to 100 mm Hg. The negative pressure applied to the surface of the skin was -120 mm Hg . It is very doubtful whether this pressure is transmitted without loss through the surface of the skin. It is quite likely that during vasoconstriction the effective negative pressure which reaches the walls of the blood vessels could be easily withstood by vessels capable of resisting internal pressures of 50 to 100 mm Hg. Under these circumstances, therefore, in the absence of an adequate reservoir for the accommodation of blood flowing through the arterioles it would be expected that with high vasoconstrictor tone the effectiveness of suction and pressure would be diminished if not completely absent in some instances. The effect of immersing one forearm in warm water (Figs 4A and 4B) is in favor of this explanation. vasoconstrictor tone was reduced slightly a reservoir.

TABLE 4—(Continued)

Observation number	Subject	Room temperature	Digit	Skin temperature		Duration of pressure and suction (left only)	Average temperature of air in boxes		Changes in skin temperature during suction and pressure	State of subject
				At beginning of period of suction and pressure	At end of period of suction and pressure		Beginning of pressure changes	End of pressure changes		
A32†	G	C 10.6-16.0	L1 L3	C 21.9 22.4	C 28.4 26.6	minutes 48	C 22.4	C 24.4	C +6.5 +4.2	Chilly
				28.3 22.6	25.5 23.6	None	24.1	26.7	-2.8 +1.0	
				20.3 19.9	21.2 20.6	56	19.8	20.2	+0.9 +0.7	
A1	G	14.2-15.8	R1 L1 L3	22.4	22.5	None	21.5	21.3	+0.1	Cold shivering
				19.0 18.8	18.5 18.0	49	15.2	15.1	-0.5 -0.8	
				25.2	20.4	None	15.3	16.3	-4.8	
A2	L	8.9-11.8	L1 L3 R1	19.5 19.2	20.1 19.6	54	17.2	19.1	+0.6 +0.4	Quite chilly
				20.6	20.3	None	20.9	20.3	-0.3	
				13.6-16.3						
A3	G	13.6-16.3	L1 L3 R1	19.5 19.2	20.1 19.6	54	17.2	19.1	+0.6 +0.4	Cool
				20.6	20.3	None	20.9	20.3	-0.3	
				13.6-16.3						
A5	G	13.9-15.2	L1 L3 R1	19.5 19.7	18.6 18.9	40	19.3	19.1	-0.9 -0.8	Cold chilly
				23.5 22.4	23.3 22.7	None	21.6	21.5	-0.2 +0.3	

* Negative pressure, 160 to 180 mm Hg for periods of 25 seconds during the last 13 minutes

† Negative pressure, 170 to 180 mm Hg for periods of 18 seconds, with positive pressure, 70 to 80 mm Hg for periods of five seconds



suction and pressure favored blood flow through the left extremity, as shown by the greater rise in skin temperature. The results suggest that the maximal effects of alternate suction and pressure can be obtained only if vasoconstrictor tone is not too great.

3 The effects of suction and pressure on the vasodilator response to immersing one forearm in warm water

Gibbon and Landis (3) showed that immersing one or two forearms in warm water produced vasodilatation, more or less complete in grade, in the lower extremities. In agreement with Lewis and Pickering (4) it was observed that in general the vasodilator response began first in the warmer extremity and that the final temperature reached at the end of 35 minutes was slightly higher in the extremity originally the warmer.

The effects of suction and pressure on the vasodilator response were studied, in order to determine whether blood flow was modified by external pressure changes. Control observations were made in which the vasodilator response was induced in the lower extremities without the use of suction and pressure (Fig. 5). The left extremity was placed in the aluminium box, the right in the control box, the air temperature in the latter was kept uniformly higher than that in the aluminium box. In all observations the right or control extremity was slightly warmer at the time the forearm was immersed in the warm bath. The forearm remained in the bath for 35 minutes.

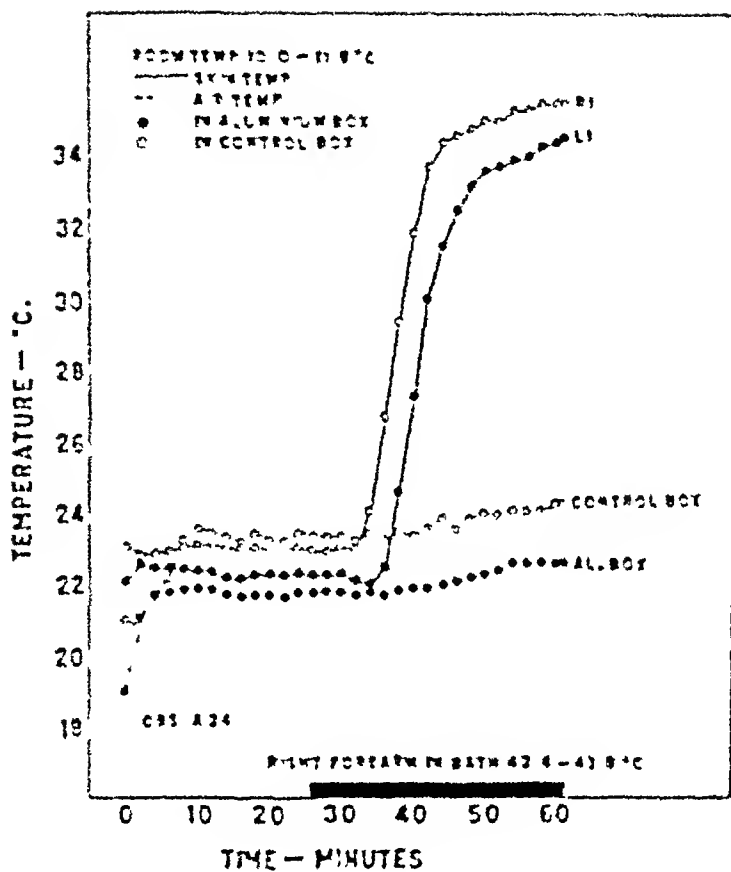
In Table 5A six such control observations are summarized. The average temperature of the air in the boxes was computed as in the previous tables. In the third column from the right the temperature of a digit was regarded as having begun to rise when it was 1°C above the temperature existing when the forearms were immersed. In agreement with previous observations the right, or warmer, digits, with one exception, showed the vasodilator response sooner than the corresponding left, or cooler, digits. In the last two columns of Table 5A the temperatures of the corresponding digits are compared as in previous tables. At the time the forearm was immersed in warm water, the left digits were the cooler by 0.4 to 1.7°C with an average difference of 1.1°C . At the end of the 35 minute immersion period the same relationship (with one exception) existed, the left digits remaining the cooler throughout with an average difference (including the one exception) of 1.8°C . This agrees with previous findings and serves as a control for those observations in which suction and pressure were used in combination with warming of one forearm for 35 minutes.

Figures 4A and B show that suction and pressure favored the development of the vasodilator response in the lower extremity exposed to pressure variations. In one observation (Fig. 4A) the warmer control extremity showed no vasodilator response whatever when the right forearm was immersed in warm water while the left extremity exposed to suction and

B With application of suction and pressure to left lower extremity during immersion of forearm in warm bath

Observation number	Subject	Room temperature	Digit	Skin temperature			Average temperature of air in boxes		Time between immersion and beginning of rise in temperature	Difference in skin temperature	
				At beginning of immersion period	At end of immersion period		Beginning of immersion period	End of immersion period		Beginning of immersion period	End of immersion period
A2	L	C 89-118	L1	C 185	C 311		C 152	C 162	minutes 24	C -19	C +117
			L3	180	307				26		
			R1	204	194		154	169	No rise		
A3	G	137-150	L1	197	339						
			L3	192	323		190	195	12	-08	+33
			R1	205	306		208	214	16		
A5	G	139-152	L1	186	337						
			L3	189	327		187	182	14	-48	+04
			R3	234	333		215	211	14	-40	+19
A9	G	131-147	L1	194	333						
			L3	187	311		190	201	14	-15	+47
			R3	209	286		203	226	24	-17	+68
A20*	L	140-158	L1	204	330						
			L3	209	315		226	237	7	-24	+18
			R3	228	299		244	258	15	-19	+16
A23*	L	120-138	L1	194	336						
			L3	199	326		212	225	9	-24	+93
			R3	218	243		234	247	8	-17	+90
				216	236				20		
									Average	-23	+51

* Extremities colder than air in box when observation was started



The difference between the control and experimental observations can be summarized in the form of averages. Without suction and pressure the left extremity, originally cooler, began to show a vasodilator response 20 minutes after the forearm was immersed while the right, being warmer, began to show the vasodilator response in 15 minutes. The left extremity, originally 1.1°C cooler than the right, remained cooler by 1.8°C even at the height of vasodilatation. On the contrary, when suction and pressure were applied to the left lower extremity the vasodilator response appeared first in the cooler (left) extremity 15 minutes after the forearm was immersed, and in the warmer (right) extremity 20 minutes after the forearm was immersed in warm water. Moreover, while the left lower extremity was cooler than the right by 2.3°C at the beginning of the immersion period it became warmer than the right by 5.1°C at the end of the immersion period when suction and pressure were used. Suction and pressure therefore reversed the findings observed in control experiments—further evidence that negative pressure favors increased blood flow. The control observations rule out the possibility that the differences were due to chance variations in the reactions of the two extremities.

C The effects of alternate suction and pressure on skin temperature in patients with peripheral vascular disease

Five patients with varying grades of peripheral vascular disease were studied. In each patient alternate suction and pressure produced a conspicuous rise of surface temperature in the affected extremity, even in the presence of advanced organic vascular disease. In these observations the room temperature was kept between 14.0 and 19.5°C —cool enough to keep air temperature within the boxes moderately low but not so cold as to be uncomfortable. The patients were in the semi-recumbent position on a couch with a back rest. One lower extremity was placed in the aluminium box while the other was placed in the control box. As usual, the air in the latter box was kept slightly warmer than the air in the aluminium box. The patients experienced no discomfort during the procedure, even falling asleep in one instance while suction and pressure were applied. Skin and air temperatures were measured in the manner described for normal subjects.

Patient 1, a white male, aged 61, was known to have suffered from diabetes mellitus since the age of 56. His blood sugar was well controlled by diet alone. For approximately two years the patient had noticed numbness, extreme coldness and moderate cyanosis of both feet without intermittent claudication or ulceration. Examination revealed generalized arteriosclerosis with a blood pressure of 155 mm Hg systolic and 85 mm Hg diastolic. On numerous occasions both lower extremities were found to be cold and slightly to moderately cyanotic in color. The skin of the right lower extremity from the middle of the leg downward was thickened and scaly and all the toe nails were abnormally ridged and thickened. Neither

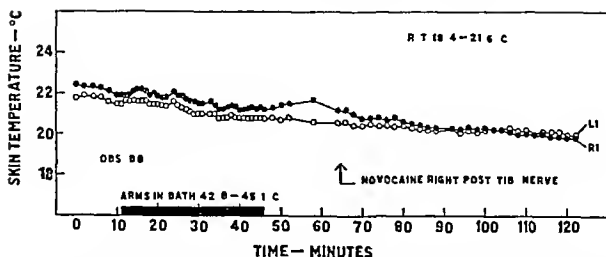


FIG 6A SHOWING COMPLETE ABSENCE OF VASODILATOR RESPONSE IN PATIENT 1 DURING (1) IMMERSION OF ARMS IN WARM WATER AND (2) DURING ANESTHETIZATION OF THE RIGHT POSTERIOR TIBIAL NERVE

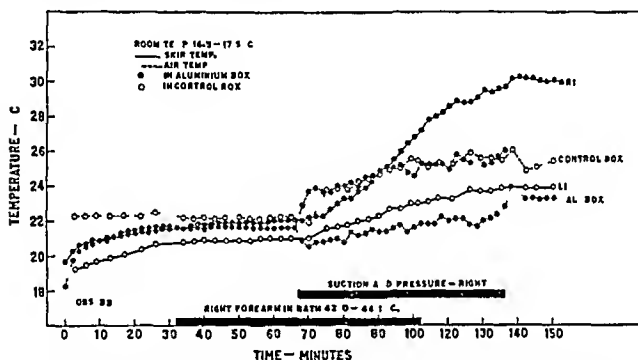


FIG 6B SHOWING RISE IN SKIN TEMPERATURE OF THE RIGHT LOWER EXTREMITY DURING APPLICATION OF SUCTION AND PRESSURE

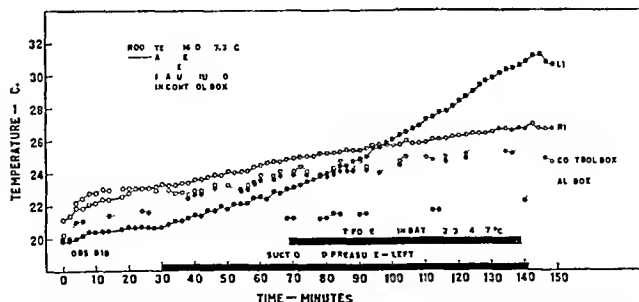


FIG 6C SHOWING RISE IN SKIN TEMPERATURE OF LEFT LOWER EXTREMITY DURING APPLICATION OF SUCTION AND PRESSURE

TABLE 6—(Continued)

Patient number	Observation	Room temperature	Digit	Skin temperature		Duration of suction and pressure	Average temperature of air in boxes		Amount of warming	Rise in skin temperature
				At beginning of period of suction and pressure	At end of period of suction and pressure		Beginning of pressure changes	End of pressure changes		
3	B20	C 14.9-16.0	R1	C 20.0	C 32.3	minutes	C	C		C 12.3
			R3	21.7	27.4	56	22.9	24.9		5.7
			L1	21.2	24.0	None	25.3	27.0	None	2.8
			L3	21.7	23.8					
4	B15	15.7-18.0	R1	27.1	31.5	27	21.6	22.3	None	4.4
			R3	26.4	30.0					
			L1	31.1	30.4	None	24.2	24.6	None	-0.7
			L3	29.5	29.1					
5	B2	16.0-17.0	R1	24.0	30.3	118	23.5	25.8	One forearm	6.3
			R3	24.4	32.3					
			L1	24.4	28.4	None	24.6	27.0		4.0
			L3	24.9	25.9					
5	B7	18.2-19.0	R1	26.6	29.4	53	25.1	25.6	None	2.8
			R3	26.8	31.4					
			L1	31.2	29.6	None	26.6	27.5		-1.6
			L3	29.0	28.4					
5	B17	14.4-16.0	R1	22.2	24.9	51	20.8	22.1	None	2.7
			R3	22.7	24.0					
			L1	22.6	23.4	None	22.4	24.3		0.8
			L3	23.0	23.2					
5	B21	14.0-15.1	R1	19.5	32.1	81	20.0	21.9	One forearm	12.6
			R3	19.2	31.7					
			L1	19.9	23.0	None	22.4	24.3		3.1
			L3	19.6	21.9					

posterior tibial nerve and the right first, third and fifth digits, originally 22.1, 21.6, and 20.5° C warmed to 32.5, 32.9 and 31.7° C respectively in a room at 19 to 20° C. This response indicated that the symptoms were due primarily to arterial spasm.

At a room temperature of 14.9 to 16.0° C the right lower extremity was placed in the aluminium box, the left lower extremity in the control box. Suction and pressure were applied to the right lower extremity for 56 minutes. At the beginning of this period the right first and third toes were slightly cyanotic and slightly cooler than the left toes. During the period of pressure changes the right digits became definitely pink and warmer by 12.3 and 5.7° C while the left digits became warmer by 2.8 and 2.1° C, the latter being exposed in the control box to an average air temperature over 2° C higher than that in the aluminium box. The right digits exposed to suction and pressure, became conspicuously warmer than the surrounding air while the left digits originally cooler than the surrounding air remained so throughout the observation.

Patient 4 (B1), a white male aged 46, was a diabetic whose right second toe had previously been amputated on account of gangrene. The tip of the right third and fourth toes had been the seat of gangrenous ulcers which healed with rest and local heat. On two occasions immersing the forearms in warm water failed to produce vasodilator response, indicating marked organic obstruction, verified in the left foot by the absence of vasodilatation after anesthetizing the left posterior tibial nerve. This patient's history has already been summarized in another paper (3).

The right lower extremity was placed in the aluminium box, the left in the control box. The temperature of the left foot was higher than that of the right both extremities being relatively warm because the patient had been brought straight from the ward where the feet were kept constantly under a warm cradle. Suction and pressure were applied to the right lower extremity for 27 minutes and during this period the right first and third digits became 4.4 and 3.6° C warmer while the corresponding digits of the other foot became 0.7 and 0.4° C cooler respectively, though the latter were exposed to environmental temperature over 2° C higher. The right digits originally cooler than the left, became, during suction and pressure, warmer than the left.

Patient 5, a white Hebrew male, aged 58, suffered from thromboangitis obliterans with coldness and cyanosis of the right foot as well as typical intermittent claudication in the right leg. The left foot became cold easily but cyanosis and pain were absent.

The posterior tibial pulse was absent bilaterally, the dorsalis pedis pulse was absent on the right but present on the left. Immersing both forearms in warm water on several occasions indicated uniformly that the arteries of the right extremity were organically diseased to a conspicuous degree while those of the left extremity were less conspicuously affected. The vasomotor index of the right foot was 1.1, that of the left 1.3.

observation charted in Figure 7A the left extremity was exposed to room air having a temperature of 18.8 to 20.1° C, the right lower extremity being placed in the aluminium box with an air temperature of approximately 24° C. Immersing one forearm in warm water was followed by a relatively rapid warming of the left first toe to 32.2° C while the right first toe in spite of the higher temperature of the surrounding air warmed more slowly to 28.1° C during the same period.

The effects of suction and pressure on skin temperature in this patient are shown in Figure 7B. The left and more nearly normal extremity was placed in the warmer control box. The right, more diseased extremity was placed in the cooler aluminium box. Suction and pressure alone failed to change skin temperature appreciably at the relatively low room temperature of 14.0 to 15.1° C. The right forearm was immersed in warm water and after approximately twenty minutes the temperature of the right first toe rose above that of the left and finally reached 32.1° C. The left first toe though originally the warmer, and in a warmer environment throughout the observation, remained cooler by 9.5° C. Four observations on this patient are summarized in Table 6. In each instance the right digits, originally cooler than the left, became warmer than the left during suction and pressure and the ordinary temperature relationship shown in Figure 7A in response to heat alone was reversed.

If all the figures in Table 6 are summarized in the form of averages it is found that the digits exposed to suction and pressure became warmer by 7.1° C while the control digits became warmer by 1.9° C although the latter were in a warmer environment. From these averages and from the individual observations it appears that applying suction and pressure increases the flow of blood to the digits even in the presence of organic arterial disease. The findings in Patients 1, 2 and 4 indicate that suction and pressure may increase blood flow though removal of vasoconstrictor tone fails to do so.

D. Skin color during suction and pressure

In this paper emphasis has been laid upon changes in skin temperature since they seemed theoretically to provide the most objective and convincing means of identifying changes in arterial blood flow. Skin color was also observed but drawing definite conclusions on this basis alone did not seem justifiable for several reasons. When the extremities are cold as emphasized by Lewis (6) in his studies of Raynaud's disease, cyanosis develops relatively slowly. As regards blood flow redness or cyanosis of the skin with surface temperature of 30° C has an entirely different significance from the same grade of redness or cyanosis at a surface temperature of 20° C. Moreover, skin color depends to a certain extent, as shown by Goldschmidt and Light (7), upon the amount of blood in the skin. Therefore, diminution of cyanosis was regarded as corroborative but not

and pressure to the left extremity was accompanied by a lesser cyanosis in the left first and third digits as compared to the right first and third digits.

In Patient 2 immersing both forearms in warm water produced neither a lessening of cyanosis nor an increase in skin temperature in the lower extremities. Negative and positive pressures caused the right first digit and the dorsum of the right foot to become definitely pink though both were cyanotic before the pump was started. An area of skin over the first metatarso phalangeal joint though deeply cyanotic before the application of suction and pressure was normal in color at the end of the observation, while the corresponding area in the left foot was still cyanotic.

In Patient 3 the right first digit, moderately cyanotic before the application of suction and pressure, was definitely pinker at the end of the observation. In Patient 4 no color observations could be made due to unsatisfactory light.

In Patient 5 the skin color of the right digits, originally moderately cyanotic, changed but little when vasoconstrictor tone was reduced by immersing both forearms in warm water. In four observations (B2, B7, B17 and B21) the color of the right digits became brighter, changing from a moderate or marked cyanotic tint before suction and pressure to a normal tint or at most a slightly cyanotic tint at the end of the suction and pressure period.

The observations on skin color corroborated the more objective and definite skin temperature studies both indicating an increased blood flow during the application of alternate suction and pressure.

DISCUSSION

The desirability of increasing local blood supply in certain medical and surgical conditions involving the extremities has long been recognized. As an early attempt in this direction the application of a bandage proximal to the lesion was, according to Bier (8), first described by Pare (9). This method was later used chiefly for treating ununited fractures by Nicoladoni (10), Thomas (11), and Helferich (12) but has been more recently revived and extended by Bier (8). While such passive congestion produces venous engorgement and increases the amount of blood trapped in the vessels distal to the bandage it does not increase, but really reduces total blood flow if congestion is extreme, as shown by the calorimetric measurements of Stewart (13).

Negative pressure was apparently first extensively used in 1829 by Junod (14) who constructed a series of 'pneumatic chambers' designed to accommodate one or more extremities or even most of the body. Under the name of 'hemospasie' negative pressure was used enthusiastically to treat almost every form of local or systemic disease. Junod used negative pressures amounting to between $\frac{1}{2}$ and $\frac{1}{4}$ atmosphere over more or less

tightness of the skin when vasoconstrictor tone was reduced by warming the forearms. When vasodilatation was not extreme 25 seconds of negative pressure produced little sensation of distention. In all of the observations on normal subjects and patients discomfort has been negligible and no hemorrhages into the skin have been observed. A negative pressure of 120 mm Hg would undoubtedly produce cutaneous hemorrhages if it were permitted to act for a long time. However, during periods of 25 seconds the veins and capillaries are not maximally distended and consequently the vessel walls are not stretched to a point where hemorrhages are apt to occur. Moreover, in patients with organic arterial obstruction distention would occur at a later period than in normal subjects whose arteries are still of normal calibre.

The cuff congests the leg slightly and in itself tends to keep the veins distended. Hence brief periods of pressure were used to empty the peripheral vessels and to provide an adequate reservoir for the accommodation of the blood drawn in by the succeeding period of suction, an essential point theoretically in favoring an increase in the rate at which arterial blood enters the capillaries and veins. Simple intermittent suction with rest periods would probably be less efficient since (a) the congestion produced by the cuff would not be overcome and (b) emptying would be slow and incomplete. The importance of diminishing vasoconstrictor tone has already been considered.

Inspection of the tables will show that occasionally skin temperatures were lower than the temperature of the air in the boxes. At the outset the air in the boxes had the same temperature as room air. As soon as the boxes were closed the air surrounding the extremities began to warm presumably due to radiation from the warmer proximal portions of the extremity enclosed within the boxes. Nevertheless, the temperature of the digits exposed to suction and pressure rose well above environmental temperature.

The possibility that the rise in skin temperature was due purely to physical forces incident to changing air pressure can be ruled out by inspection of Figures 4A, 4B, 6C, and 7B. If the elevation of skin temperature had been due to condensation of moisture or to heat produced during compression of the air within the box the skin temperature would have begun to rise in every instance as soon as the pressure variations were used. In these four observations as well as many others skin temperature did not change, in spite of suction and pressure, until vasoconstrictor tone was diminished. Moreover, such physical effects could not explain the elevation of skin temperature above the environmental temperature. A bottle covered with moist gauze the latter to simulate moist skin, cooled slightly in the aluminium box during the prolonged application of positive and negative pressure. These observations are believed to rule out artefacts due to purely physical factors.

tightness of the skin when vasoconstrictor tone was reduced by warming the forearms. When vasodilatation was not extreme 25 seconds of negative pressure produced little sensation of distention. In all of the observations on normal subjects and patients discomfort has been negligible and no hemorrhages into the skin have been observed. A negative pressure of 120 mm Hg would undoubtedly produce cutaneous hemorrhages if it were permitted to act for a long time. However, during periods of 25 seconds the veins and capillaries are not maximally distended and consequently the vessel walls are not stretched to a point where hemorrhages are apt to occur. Moreover, in patients with organic arterial obstruction distention would occur at a later period than in normal subjects whose arteries are still of normal calibre.

The cuff congests the leg slightly and in itself tends to keep the veins distended. Hence brief periods of pressure were used to empty the peripheral vessels and to provide an adequate reservoir for the accommodation of the blood drawn in by the succeeding period of suction, an essential point theoretically in favoring an increase in the rate at which arterial blood enters the capillaries and veins. Simple intermittent suction with rest periods would probably be less efficient since (a) the congestion produced by the cuff would not be overcome and (b) emptying would be slow and incomplete. The importance of diminishing vasoconstrictor tone has already been considered.

Inspection of the tables will show that occasionally skin temperatures were lower than the temperature of the air in the boxes. At the outset the air in the boxes had the same temperature as room air. As soon as the boxes were closed the air surrounding the extremities began to warm presumably due to radiation from the warmer proximal portions of the extremity enclosed within the boxes. Nevertheless, the temperature of the digits exposed to suction and pressure rose well above environmental temperature.

The possibility that the rise in skin temperature was due purely to physical forces, incident to changing air pressure can be ruled out by inspection of Figures 4A, 4B, 6C, and 7B. If the elevation of skin temperature had been due to condensation of moisture, or to heat produced during compression of the air within the box the skin temperature would have begun to rise in every instance as soon as the pressure variations were used. In these four observations as well as many others skin temperature did not change, in spite of suction and pressure, until vasoconstrictor tone was diminished. Moreover, such physical effects could not explain the elevation of skin temperature above the environmental temperature. A bottle covered with moist gauze the latter to simulate moist skin, cooled slightly in the aluminium box during the prolonged application of positive and negative pressure. These observations are believed to rule out artefacts due to purely physical factors.

BIBLIOGRAPHY

- 1 Landis, E M, and Gibbon J H, Jr, *J Clin Invest*, 1933 **xii**, 105 The Effects of Temperature and of Tissue Pressure on the Movement of Fluid through the Human Capillary Wall
- 2 Lewis, T, and Grant, R, *Heart*, 1925, **xii**, 73 Observations upon Reactive Hyperaemia in Man
- 3 Gibbon, J H Jr, and Landis, E M, *J Clin Invest*, 1932 **xi**, 1019 Vaso dilatation in the Lower Extremities in Response to Immersing the Fore arms in Warm Water
- 4 Lewis, T, and Pickering, G W *Heart*, 1931 **xvi**, 32 Vasodilatation in the Limbs in Response to Warming the Body with Evidence for Sympathetic Vasodilator Nerves in Man
- 5 Lewis, T, *Heart* 1924 **xi**, 109 The Force Exerted by the Minute Vessels of the Skin in Contracting
- 6 Lewis, T, *Heart* 1929-30 **xv**, 7 Experiments Relating to the Peripheral Mechanism Involved in Spasmodic Arrest of the Circulation in the Fingers a Variety of Raynaud's Disease
- 7 Goldschmidt, S and Light, A B, *Am J Physiol*, 1925, **lxxiii** 173 A Cyanosis, Unrelated to Oxygen Unsaturation, Produced by Increased Peripheral Venous Pressure
- 8 Bier, A Hyperemia as a Therapeutic Agent (Translation by G M Blech), 1915
- 9 Pare, d'A Oeuvres Completes (Translation of Malgaigne 1840, Vol II, Book **XLIII** p 346) Balliere, Paris, 1840
- 10 Nicoladoni, K *Wien med Wchnschr* 1875 **xcv** 81, 109 and 124 v Dumreicher's Methode zur Behandlung drohender Pseudarthrosen
- 11 Thomas H O Contributions to Surgery and Medicine Part VI, Lewis London, 1886
- 12 Helferich *Arch f klin Chir*, 1887, **xxxi**, 873 Ueber künstliche Vermehrung der Knochenbildung
- 13 Stewart, G N *Arch Int Med*, 1917 **xi** 335 The Effect of Bandaging of the Legs on the Rate of Blood Flow in the Feet
- 14 Junod V I *Traite theorique et pratique de L'Hemospasie* Masson Paris, 1875

PROCEEDINGS OF THE TWENTY-FIFTH ANNUAL MEETING
OF THE AMERICAN SOCIETY FOR CLINICAL IN-
VESTIGATION HELD IN WASHINGTON,
D C, MAY 8, 1933

Treatment of Pellagra with Liver Extracts By DAVID T SMITH and (by invitation) JULIAN M RUFFIN, Durham N C

During the past two years a series of pellagra patients have been studied at the Duke Hospital. A basic diet was planned which contained all the normal necessary vitamins and minerals except vitamin B2 or G. The patients maintained on this diet failed to make satisfactory improvement until vitamin G in the form of liver extract was added to the diet. It has been demonstrated that the symmetrical skin lesions in pellagra are due to symmetrical exposure to sunlight and have no relation to nerve lesions in the spinal cord. Unilateral skin lesions could be produced at will by proper exposure to sunlight. Experimental black tongue in dogs has been produced with the same diet used as a basic diet for the clinical studies. This experimental black tongue can be cured by liver extracts.

The Urinary Excretion of Iodine By GEORGE M CURTIS and (by invitation) FRANCIS J PHILLIPS, Columbus, Ohio

The urinary excretion of iodine by hospital patients without thyroid disease in this region is from 25 to 75 microgrammes daily. No iodized salt is used in the hospital diet. It is within normal range in patients with non toxic nodular goiter and also in those with non toxic diffuse colloid goiter. In patients with diffuse hyperplastic goiter with exophthalmos and with severe hyperthyroidism, it is greatly increased during the early exacerbation of severe, untreated toxicity. The urinary excretion of iodine increases greatly immediately following thyroidectomy. This increase is temporary. When iodine is quantitatively administered to patients with diffuse hyperplastic goiter with hyperthyroidism, the urinary excretion is at first low. There ensues a rising iodine excretion curve as clinical improvement follows. The urinary excretion of iodine is increased during early menstruation. This is correlated with the associated rise in the blood iodine.

Congenital Heart Block. Review with Report of Detailed Histopathologic Study of the Second Case of Complete Congenital Heart Block Studied by Serial Sections Through the Conduction System. By WALLACE M YATER and (by invitation) JAMES A LYON and PAUL E McNABB
Washington D C

Cases of congenital heart block are not common. About forty cases have been reported in the literature which fulfill certain arbitrary criteria for the making of this diagnosis. The cause has usually been patent interventricular septum with anatomical break in the atrial septum. However, necropsy findings of only four cases have been reported. In only two were studied by serial sections through the conduction system. In one was a case of 2 to 1 heart block in which there was almost complete interruption of the interventricular septum. The other was a case studied by Yater

heart block in which there were very unusual anomalies but in which a septal defect did not play a rôle. The case now reported is one of an infant who had a defect in the interventricular septum in its middle upper portion. There was found a complete interruption of the bundle of His by the defect. In fact, practically the whole bundle was missing. The electrocardiogram, however, showed the QRS complex to be of the supraventricular type. The diagnosis of complete heart block due to patent interventricular septum was made during life.

Significance of Inorganic Sulphate Coefficient or Clearance in Renal Disease

By JOHN W. MACY (by invitation) and NORMAN M. KEITH, Rochester, Minn.

Inorganic sulphate concentration was determined in blood serum and urine during a given period of urine excretion. The coefficient or clearance was determined by the following formula

$$\frac{\text{Per cent sulphate concentration in urine}}{\text{Per cent sulphate concentration in blood serum}} \times \text{minute volume of urine}$$

In twelve normal individuals the sulphate coefficient or clearance was very constant from hour to hour, the variation being less than in the corresponding urea and creatinine coefficients. This fact suggests that the inorganic sulphate coefficient might be useful as a standard with which the coefficient of other substances might be satisfactorily compared.

In renal disease the sulphate coefficient is in general lowered in proportion to the degree of insufficiency. In some cases of this series, the sulphate coefficient appears to be decreased when other functional tests, including urea clearance, are within normal range. This suggests that the sulphate coefficient or clearance may have an important place in those cases in which early renal insufficiency is suspected.

The Effect of Gelatin Diets in Nephritis By G. P. GRABFIELD, Boston, Mass.

Previous studies of nephritis have indicated that there is some alteration in patients with Bright's disease in their handling of sulphur, more especially in relation to the nitrogen. Therefore, in the present study it was decided to try the effect of sulphur-poor diets on patients with Bright's disease. The problem of providing adequate amounts of nitrogen without sulphur was solved by using gelatin as the source of practically all the dietary nitrogen. In practice, standard nephritic diets were used, patients were studied for three-day periods and during the test period, practically all the protein of the diet was given to the patient in the form of gelatin in the same amount as the previous protein of the diet. These diets were difficult to take and the only cases reported are those which succeeded in eating all the diet.

Unfortunately, few control experiments of this type are recorded in the literature but those that are, indicate that gelatin is an incomplete protein and is only partly utilized. The degree of utilization varies and the estimates are that from 11 to 40 per cent must be made up by the breakdown of body protein. The interesting thing about the figures as shown, is that if we take the sulphur as an index of the amount of nitrogen metabolized, it remains essentially the same during the gelatin periods as previously. As these diets were adequate except for the protein and sulphur, it seems to us that this may be taken as an indication that there is an alteration in the utilization of sulphur in these patients with Bright's disease. In other words, these figures indicate that the

amount of the sulphur rich moiety of the protein molecule that is metabolized in such patients is only partly dependent upon the diet

The Effects of Posture (Standing) on the Serum Protein Concentration and Colloid Osmotic Pressure of Blood from the Foot in Relation to the Formation of Edema By JOHN B. YOUNG and (by invitation) H. S. WELLS, DOROTHY DONLEY, and D. G. MILLER, Nashville, Tenn.

The influence of posture on the serum proteins and osmotic pressure of blood in the foot was studied with reference to formation of edema, particularly in persons with slightly lowered serum proteins. Others have shown a general concentration of blood in the erect posture, apparently due to loss of fluid from the vessels in the dependent tissues but protein concentration and osmotic pressure of blood from the foot have not been determined. Normal subjects after standing approximately an hour showed a 17 to 40 per cent concentration of proteins and a 24 to 64 per cent increase in osmotic pressure in blood from the foot. In all cases the rise in osmotic pressure was relatively greater than the concentration of protein. Concentration was much greater in the foot than in the arm. The escape of fluid was indirectly indicated by an increase in leg volume but pitting edema was absent in most normals. Patients with edema showed an increase in proteins of 14 to 33 per cent and in osmotic pressure of 23 to 46 per cent but the average increases were less than in normals while the increase in leg volume was greater and the edema was demonstrably increased.

A Study of the Nasal Secretions in Disease of the Upper Respiratory Tract By PERRIN H. LONG and (by invitation) ELEANOR A. BLISS and HARRIET M. CARPENTER, Baltimore, Md.

Studies of the nasal secretions during "colds" have shown that in the early hours of a "cold" many epithelial and mononuclear cells are found. By the third day the cellular content of the nasal secretions is chiefly made up of polymorphonuclear leukocytes. In many instances stained slide preparations of the nasal secretions late in "colds" failed to show appreciable numbers of bacteria. It is suggested that the cellular reaction of the nasal secretions in "colds" is a response to the infecting filterable agent rather than to the bacterial flora of the nasal passages.

Contraction of the Spleen in a Case of Anemia with Splenomegaly By D. K. MILLER (introduced by C. P. Rhoads) New York City, N. Y.

Contraction of the spleen was studied during the intravenous injection of liver extract in a patient with anemia and splenomegaly. This afforded an opportunity to ascertain certain facts regarding the function of the human spleen as a reservoir of red and white corpuscles and blood platelets.

The spleen was visualized by x-ray. Its area was measured before and after the injection of liver extract. The contraction occurred during the injection, which was given over a period of twenty minutes. The spleen regained its original size after one half to six hours.

In a typical study the surface area of the spleen as measured from the x-rays with a Keuffel and Esser planimeter was 903 sq. cm. before the injection. Immediately after the injection the surface area was 323 sq. cm. Twenty-four hours later the area was 891 sq. cm. Accompanying this contraction there was an increase in the red cell count of 151 per cent. The hemoglobin increased 21.5 per cent, and the white cell count 54.0 per cent. An

increase in the number of blood platelets of 120 per cent occurred. The fragility of the red cells to sodium chloride solution was not altered.

Blood volume determinations by the vital red method of Rowntree showed no appreciable alteration. There was a decrease in the plasma percentage of 77 per cent.

From data of this nature it has been possible to estimate the relative cellular composition of the blood given off from the splenic reservoir into the general circulation as a result of induced and observed contractions of the spleen.

The Nature of the Muscular Weakness in Graves' Disease By E. SHORR, H. B. RICHARDSON and H. G. WOLFF, New York City, N. Y.

Muscular weakness is a common symptom in Graves' disease. In such patients a very constant abnormality is the presence of excessive amounts of creatin in the urine. Creatin in muscle has been shown to be combined with phosphoric acid as a labile compound, phosphocreatin (Fiske and Subbarow), now considered to be of primary significance for muscular contraction. From experiments on certain pathological states, it appears probable that glycine is an important precursor of creatin. This amino-acid is readily synthesized in the body, chiefly in the liver.

Recently, considerable information has been obtained concerning the relation between the muscular weakness and the disturbance of creatin metabolism in progressive muscular dystrophy. Since the muscular weakness in Graves' disease is often very great, and a creatinuria is common to both conditions, the creatin metabolism of patients with Graves' disease was investigated. Several analogies between the two syndromes were revealed. Thus, in both (1) the ability to retain ingested creatin (presumably as phosphocreatin) was markedly diminished, (2) the ingestion of glycine resulted in large increases in creatin elimination, frequently greater in Graves' disease than in advanced cases of progressive muscular dystrophy. In some instances the rise was transitory, falling to lower levels or disappearing with continued glycine feeding, (3) the ability to form glycine, as measured by hippuric acid excretion after the administration of sodium benzoate, was unimpaired. Furthermore, following ingestion of sodium benzoate, there was frequently observed a sharp increase in the creatin output, the significance of which is yet unknown.

An additional point of resemblance was furnished by the observation that long-standing cases of Graves' disease showed anatomical changes in the skeletal muscle similar in many respects to those found in progressive muscular dystrophy (Askanazy).

Following the administration of iodine to patients with Graves' disease, as was previously observed (Palmer et al.), there was a progressive fall in creatinuria to the normal level, usually accompanied by a fall in the basal metabolism. During the latter phase, we gave creatin and glycine again, but now no increase in creatin output resulted. This is analogous to what occurs in progressive muscular dystrophy after prolonged feeding of glycine. Additional ingestion of glycine and creatin then produces no increase in creatin output (Thomas, Milhorat and Techner).

The factors causing these disturbances of creatin metabolism in Graves' disease are still obscure. It is postulated that the processes maintaining the integrity of the phosphocreatin mechanism are taxed too severely by the persistently high metabolism. One of these, the endogenous formation of glycine, was found to be normal. However, though adequate in proportion to the body weight and the normal basal metabolism, its production may be low in proportion

to the needs of an elevated total metabolism. The increased demands resulting from the latter may lead to a progressive depletion of the elements involved in the phosphocreatin system. Whether iodine acts directly on this muscle mechanism in addition to reducing the basal metabolism, cannot be stated.

Conclusion. The muscular weakness in Graves' disease is the result of a reparable impairment of the phosphocreatin mechanism, and is of the nature of an acute muscular dystrophy similar in many respects to the disturbance in progressive muscular dystrophy.

The Effect of Convulsant Drugs on the Cerebral Vessels. By STANLEY COBB and (by invitation) JACOB E. FINESINGER, Boston, Mass.

This paper is a report of microscopical observations through a window in the skull of the small arteries of the pia, and of their reactions after the administration of convulsant drugs to the animal under observation. The drugs used were caffeine, wormwood oil, homocamfin, camphor monobromate and picrotoxin. The intravenous introduction of large doses of caffeine produced an acute constriction of the pial arteries followed by a generalized convulsion. Small doses of wormwood oil gave a slight constriction of the pial artery followed by a generalized convulsion. Large doses of wormwood oil produced a dilatation of the pial arteries before the onset of the generalized convulsion. Homocamfin and picrotoxin produced a slight constriction of the pial artery preceding the convulsion. Camphor monobromate gave a dilatation of the pial arteries preceding the convulsion. These experimental convulsions were not preceded by a consistent type of vascular response to the drugs used.

The Nonprotein Iron of the Blood. By J. F. MCINTOSH, Peiping, China.

Relatively little attention has been paid to that part of the iron of the blood which is not contained in the hemoglobin molecule. Most investigators have considered it as negligible. Scarcely any determinations of it have been made in clinical cases.

The nonprotein iron of whole blood has been determined directly by analysis of the trichloroacetic acid filtrate. In normals the average value found was 1.02 mgm. per 100 cc. A variety of anemic cases were studied, including pernicious anemia, achlorhydric hypochromic anemia, and secondary anemias due to hemorrhage, cancer, and various infections. In these, the nonprotein iron was reduced in proportion to the degree of anemia. The different diseases do not show any grouping which deserves emphasis.

The greater part of the nonprotein iron is to be found in the red cells.

The Case of Captain Charles Martell: What It Has Taught Us About Generalized Osteitis Fibrosa Cystica. By WALTER BAUER and (by invitation) CHARLES L. SHORT, Boston, Mass.

The diagnosis of generalized osteitis fibrosa cystica was first made in this country by DuBois in 1926. Through the kindness and generosity of Dr. DuBois we have been privileged to study this patient, Captain Charles Martell, at various times during the ensuing six years. Because of his courage, interest in clinical investigation, and desire to live as well as to help others live, he permitted detailed clinical and metabolic studies on numerous occasions, submitted to eight operations, and asked that a complete necropsy be done at the time of his death.

A detailed review of these studies reveals not only the clinical and metabolic course of generalized osteitis fibrosa cystica, its complications, the metabolic changes resulting from these complications, but also demonstrates the difficulty that may be encountered in finding the offending parathyroid tumor in this disease, as well as the pathological changes resulting from hyperparathyroidism of fifteen years duration

The Conversion of Glycerol to Glucose by the Animal Organism By F H LASHMET (by invitation) and L H NEWBURGH, Ann Arbor, Mich

In its metabolism, 10 per cent of fat is converted to glycerol. On the basis that 100 per cent of glycerol is converted to glucose, it has been customary to consider 10 per cent of the fat of the diabetic diet as available glucose.

The data, upon which the conclusion is based that glycerol is completely converted to glucose, are very unconvincing, even as originally presented, and especially so when recalculated.

Our investigation, employing more recent methods than were available to older observers, seems to show that less than 30 per cent of glycerol is converted to glucose. If our work is substantiated, it is evident that only 2 or 3 per cent of fat is converted to glucose.

Studies on the Origin of Plasma Proteins By HOBART A REIMANN and (by invitation) GRACE MEDES and LUTHER C FISHER, Minneapolis, Minn

Previous experiments indicated that an increase of blood viscosity due to increase of globulin and fibrinogen caused enhancement of the agglutination of bacteria. Studies were then made to locate the source of these proteins which appear to be associated with the immune defense mechanism. There are four theoretical sources of fibrinogen: (a) liver, (b) erythrocytes, (c) bone marrow, (d) leukocytes. Considerable presumptive evidence favors the latter two, especially the leukocytes.

Leukocytes were obtained by injecting aleuronat or gum acacia into the pleural cavities of rabbits. The cells were disintegrated by freezing with liquid air and triturating in a mortar. The resulting viscid mass was redissolved in salt solution, dialyzed and fractionated with sodium sulfate. Proteins with the salting-out characteristics of fibrinogen, globulin and albumin were present. Immunologic tests are under way to test the identity of these proteins with their counterparts in the plasma.

Heavy suspensions of leukocytes in broth were placed in cellophan capsules and embedded subcutaneously in rabbits. After 4 to 7 days the capsules were removed. The leukocytes had largely disintegrated and globulin- and fibrinogen-like proteins were detected in the broth. Leukocytes were then destroyed *in vivo* by benzol injections or by exposure to x-rays. In one benzol experiment the globulins and fibrinogen increased markedly as the number of circulating leukocytes fell. During the period of aplasia of the marrow, the fibrinogen diminished. Other experiments failed to give striking results. In general, the fibrinogen tended to increase, and the total protein to diminish at the expense of the albumin fraction. The marked leukopenia produced by x-rays caused but little change in the blood proteins. The fibrinogen increased somewhat immediately following the diminution of leukocytes.

These experiments indicate that globulin and fibrinogen may in part be derived from the decomposition of the leukocytic cells, especially of the polymorphonuclear type in the circulation or in the hematopoietic system.

Allergy and Immunity in Tuberculosis By JONAS S FRIEDENWALD and (by invitation) HERBERT ROTHSCHILD and CLARENCE BERNSTEIN, Baltimore, Md

A group of guinea pigs was made immune to tuberculosis and allergic to tuberculin by inoculation with a virulent strain of tubercle bacilli. When allergy had been well established one half of the animals were desensitized by daily subcutaneous injections of Koch's OT in increasing doses. When allergy had been completely suppressed, the desensitized animals together with the allergic animals and a group of normal controls were inoculated in the groin, in the eye and in the skin with a virulent strain of tubercle bacilli. The injections of tuberculin in desensitized doses were continued daily. The results showed that the loss of allergy by desensitization was not accompanied by any loss of immunity.

Heterologous Scarlet Fever By JAMES D TRASK and FRANCIS G BLAKE, New Haven, Conn

Two cases of scarlet fever which failed to respond to large doses of therapeutic scarlet fever antitoxin were found to have a heterologous toxin in their blood. The blood serum from these patients collected during the active stage of the disease elicited strongly positive skin reactions in certain Dick negative test subjects and no reaction in Dick positive subjects. In vitro the serum was neutralized by the blood serum of the Dick positive individuals but not by that of the Dick negative individuals.

A toxin was prepared from the hemolytic streptococci isolated from the two patients. Both in the reactions it elicited on intracutaneous injection and by the results of neutralization tests it corresponded with the toxin in the blood samples mentioned above.

Accordingly, the above results taken in conjunction with our previous studies show that heterogeneity exists among the toxins found in the blood during scarlet fever and among the antitoxins found in the blood serum of normal individuals.

Electrocardiograms that Record the Potential Variations Produced by the Heart Beat at a Single Point By PAUL S BARKER, FRANK N WILSON and (by invitation) FRANKLIN D JOHNSTON, Ann Arbor, Mich

A method has been devised which makes it possible to record the potential variations produced by the heart beat at a single point. This method has been used to explore the electric field produced by the heart at the body surface in bundle branch block, ventricular hypertrophy, coronary occlusion and other cardiac conditions. By this means, it is possible to compare the potential variations that occur at points near the heart with the potential variations of the extremities. In general it is found that the potential variations of a given extremity are similar to those that occur on those surfaces of the heart that face toward the attachment of that extremity. In the common type of bundle branch block and in left ventricular enlargement the potential variations of the left arm are similar to those that occur over those portions of the precordium that overlie the left ventricle, the potentials of the left leg are similar to those that occur over those portions of the precordium that overlie the right ventricle. Characteristic curves are obtained from the precordium when the anterior surface of the heart is infarcted.

The Effect of Vagus and Sympathetic Stimulation on the Coronary Flow in Revived Human Hearts By WILLIAM B KOUNTZ (introduced by David P Barr), St Louis, Mo

Human hearts have been revived in the body by perfusion. The hearts were perfused through an aortic cannula until they began to beat strongly. Cannulae were then inserted into the right coronary artery and into the circumflex branch of the left coronary artery. These were then connected to a reservoir of perfusion fluid, the height of which could be regulated so as to obtain any desired pressure. In some experiments another reservoir containing Locke's solution free of calcium or with excess of calcium was arranged in the perfusion circuit. The flow of fluid from the reservoirs was measured by a volumetric recorder.

When the coronary vessels were perfused with buffered Locke's solution containing at least 15 per cent blood, stimulation of the peripheral end of the vagus nerve slowed the heart rate and increased the coronary flow. Stimulation of the sympathetic produced exactly opposite effects under the same circumstances.

Further analysis was made by stopping the heart through removal of calcium from the perfusing solution or through the addition of an excess of calcium. In some experiments changes in the pH of the perfusion fluid in the range from 7.0 to 7.8 were made.

It was found that when hearts were stopped by removal of calcium or by reducing the pH of the perfusion fluid to about 7.0 vagus stimulation had no influence on the coronary flow. Sympathetic stimulation on the other hand increased the flow. When the hearts were stopped by an excess of calcium or by increasing the pH to about 7.8 vagus stimulation increased the coronary flow, while sympathetic stimulation had no influence upon it.

In the beating heart the mechanical factor of change in heart rate makes it difficult to evaluate the effect of the sympathetic and vagus activity on the heart. It is suggested that changes in the coronary flow in the arrested hearts may be due to change in tone.

Further Observations on the Use of Chest Leads in the Electrocardiographic Study of Coronary Occlusion By CHARLES C WOLFERTH and (by invitation) SAMUEL BELLET, THOMAS M McMILLAN, and FRANCIS CLARK WOOD, Philadelphia, Pa

Fifty-seven cases of acute coronary occlusion and 280 controls, both normal and pathological, were studied with chest leads and limb leads. The following facts developed:

- 1 Twenty-three of our fifty-seven cases of acute coronary occlusion showed signs of recent cardiac infarction in an anteroposterior chest lead (Lead IV) and not in limb leads, at some time during their course.

- 2 Three types of left ventricular infarction have been seen at necropsy: the anterior (5 cases), the posterior (1 case) and the lateral (1 case). Lead IV helps to differentiate these types electrocardiographically. In our series of 57, 33 were anterior, 18 were posterior, 5 were probably lateral, and 1 was not classified.

- 3 Huge upright T-waves in Lead IV probably signify acute or subacute anterior infarction.

- 4 The advantage of Lead IV over limb leads is seen mainly in the study of cases of anterior infarction. Twenty-one of thirty-three acute anterior cases, at some time during their course, showed diagnostic findings in Lead IV and not

in limb leads. Moreover, Lead IV sometimes helps to differentiate T-1 inversions produced by healed anterior infarction from those due to other causes.

5 In our cases the pain of anterior infarction has usually been situated lower in the chest than the pain of posterior infarction.

6 Anterior infarction seems to have a less favorable prognosis for life and for adequate recovery than posterior infarction.

7 A standard normal electrocardiogram in the anteroposterior chest lead is as readily obtainable as a standard in limb leads.

8 S-T interval deviations caused by conditions other than acute coronary occlusion are if anything less confusing when chest leads are used with limb leads. They rarely resemble the typical electrocardiographic picture of either of the two common types of coronary occlusion.

Bone Lesions in Yaws By THOMAS B. TURNER and (by invitation) GEORGE M. SAUNDERS, Baltimore, Md.

Approximately 10 per cent of 1100 yaws patients studied in Jamaica exhibited lesions of the bones, although among individuals infected less than five years the incidence was 14 per cent. The characteristic lesion as revealed by roentgen ray consists of multiple small areas of rarefaction in the cortex with or without periosteal proliferation; rarely periostitis occurs alone. Often there is new bone formation about the areas of rarefaction giving rise to clinically recognizable enlargement and deformity of the bone. The long bones are chiefly affected. The onset of typical lesions has been observed as early as one month and as late as fifteen years after infection with yaws. Persistent dull pain is the most prominent symptom and active lesions may persist for several years eventually healing spontaneously or extending to involve the overlying soft parts with subsequent development of chronic ulcers. If not too far advanced there is prompt response to anti-yaws treatment. Photographs of the clinical and roentgenological appearance of typical bone lesions were shown.

Preliminary Report on the Treatment of Chronic and Subacute Infectious Arthritis by Artificial Fever By LAWRENCE A. KOHN and (by invitation) STAFFORD L. WARREN, Rochester, N. Y.

Forty-three patients in the main with purely atrophic, proliferative, and some mixed types (infectious and degenerative arthritis), in various stages of disability, deformity, and chronicity (six months to fourteen years) have been subjected to one or two treatments of artificial fever. No cases of gonorrheal origin or purely degenerative arthritis have been included. Twenty-one patients were treated in 1931; of these five have not returned in 1933 (one was not improved and the rest were improved when last heard from after 1 to 14 months). Twenty-two patients were treated in 1932 (of these two have not returned and one was unimproved one month later and has been lost sight of). The results have been quite consistent in thirty-five patients, in all but two of whom pain has rapidly diminished and mobility of joints has increased; a steady improvement in the condition of the patient; a gain in weight (2 to 17 kgm.), and disappearance of pallor have occurred in the following four to six months.

Relapses have occurred at the end of 12, 20, and 24 months respectively in three patients in whom another treatment has been successful in giving relief. Eight incapacitated patients have been able to earn a living.

Antibody Response to Pneumococcic Infections By MAXWELL FINLAND and ALEXANDER W WINKLER (introduced by Chester S Keefer), Boston, Mass

One hundred and fifty-eight patients from whom pneumococci of various types (Cooper) other than Type I were isolated were studied with reference to the formation of antibodies. Of these, 110 were patients with pneumonia and the remainder had other acute or chronic respiratory infections. Tests for serum agglutinins were carried out before and after crisis by the use of antigens prepared from a variety of type-specific pneumococci. Each serum was tested with one or more antigens of the type homologous with that recovered from the patient and, in addition, against a number of antigens of heterologous types. In the case of Types II, III, V and VIII protection tests were also carried out.

The results showed that agglutinin formation can be demonstrated in the course of pneumonia and occasionally of other infections associated with the finding of some of the new types of pneumococci. The specificity of the antibody response is similar to that commonly observed in infections with Type I pneumococci. In particular, homologous type-specific antibodies were demonstrated in patients from whom the following types were obtained: Types II, III, V, VII, VIII, IX, XII, XIV, XVII, XVIII and XIX. Among the patients with Types II and V (IIa, Avery) pneumococci, homologous type-specific antibodies were found for each type, with only occasional development of heterologous antibodies as demonstrated by mouse protection tests. With Types III and VIII (atypical III, Sugg and Harris) a similar specificity in the production of antibodies occurred, but, in addition, there was some production of Type III antibodies in pneumonias associated with Type VIII pneumococci and vice versa.

The findings, in general, tend to confirm the biological identity of the newly classified types and point toward their etiological relationship to human disease.

Digestion Efficiency in Simple Undernutrition By J M STRANG and H B MCCLUGAGE (by invitation) and FRANK A EVANS, Pittsburgh, Pa

Nine undernourished patients increased their diets to a daily average of 3475 calories on which they gained an average of 7.3 kgm in 56 weeks. The average weight of the food was 1926 grams per day. The average daily weight of the stool was 165 grams or 8.5 per cent of the food intake. This ratio varied according to the water content of food and stool. The food solids bore a fairly constant relation to the stool solids from week to week. The average weight of the food solids was 549 grams, of the stool solids, 31 grams. This represents a digestion and absorption efficiency of 94 per cent. The nitrogen in the stools averaged 1.3 gram per day, or 12 per cent of the food nitrogen.

These studies indicate an ability on the part of patients with uncomplicated undernutrition to digest and absorb sufficient food to gain rapidly.

Congenital Heart Disease Complicating Pregnancy: A Report of Sixteen Cases By BURTON E HAMILTON (by invitation) and ROBERT STERLING PALMER, Boston, Mass

Congenital heart disease is not a common complication of pregnancy. At the Boston Living-in Hospital in over 500 cases of organic heart disease, congenital heart disease comprises less than three per cent. It is found as a complication of pregnancy once in 3000 patients. This very rarity, plus the fact that the striking physical signs of congenital heart disease sometimes impress

the first observer unduly and lead him to make an unnecessarily grave prognosis and finally a practical clinical lesson which we have drawn from our experience, has prompted us to make this report. We are presenting in addition to our sixteen cases the scattered reports of fifteen cases encountered in the literature.

We feel justified in drawing the following conclusions:

1 Coarctation of the aorta is to be considered in any case of unexplained persistent hypertension. The prognosis for the mother when this condition complicates pregnancy is probably good. The fetus may die in utero, possibly due to diminished circulation to the lower half of the body.

2 Congenital complete heart block is not a serious complication of pregnancy.

3 Patent ductus arteriosus is compatible with successful childbearing, only one in eleven cases having symptoms which could be considered at all serious. We suspect that the same may be said of intraventricular septal defect if operative delivery and obstetrical manipulations in themselves likely to induce shock can be anticipated and avoided. The danger probably lies in a reversal of the left right to a right left shunt brought about by a fall in systemic pressure.

4 Experience with six pregnancies in one patient with preexisting right left shunt suggests that operative delivery or obstetrical procedures likely to induce hemorrhage or shock may cause a serious and alarming exacerbation of this shunt. However, compensatory mechanisms already present may make the danger less formidable in these cases.

Do the Thebesian Vessels Play Any Role in Supplying Blood to the Heart?

By L. N. KATZ and (by invitation) KENNETH JOCHIM and ANNE BOHN
ING, Chicago, Ill.

Wearn's contention of the auxiliary role of the Thebesian vessels has recently been questioned. We have reinvestigated the problem, using a system by which the cannulated coronary arteries and coronary sinus form a circuit distinct from the cardiac chambers and great vessels. The only communication possible between the two circuits is by way of the Thebesian vessels. Bismuth injected into the cardiac circuit appeared in the coronary circuit as evidenced by chemical analysis of the coronary sinus blood. Bacteria injected into the coronary circuit also appeared in the coronary sinus blood and were found histologically in the capillaries and coronary vessels. The Thebesian vessels can therefore carry blood from the heart chambers into the coronary circuit but the amount carried is still undetermined.

The Calorigenic Action of Thyroglobulin and its Constituents By J. LERMAN and W. T. SALTER, Boston, Mass.

It has previously been shown by us that thyroxine polypeptide is as active when given by mouth as when given parenterally, and that its activity is proportional to its iodine content when compared with crystalline thyroxine. Using the metabolic response of untreated myxedema patients to thyroxine polypeptide given in dosage of one milligram by mouth daily as a standard we find in three assays of whole thyroid gland with an equivalent thyroxine content and in four assays of whole thyroid gland with an equivalent total organic iodine content that the calorigenic action of whole thyroid gland depends upon its total organic and not upon its thyroxine (active) iodine content.

In another study 20 patients with treated myxedema were given in succession thyroid gland preparations from different sources for periods of time sufficient to determine the maintenance level of metabolism for each 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 days.

The results indicate that the ration of a preparation of thyroid which will maintain a definite level of metabolism in a given patient or change this level is dependent upon its total organic iodine and not its thyroxin (active) iodine content. Since diiodotyrosin (the soluble iodine fraction in iodothyroglobulin) is inactive calorigenically when given alone, there remains to be explained the mechanism by which it assumes calorigenic properties when linked with other amino-acids in iodothyroglobulin

One patient with myxedema was fed thyroglobulin obtained from hyperplastic glands, two others were fed thyroglobulin obtained from colloid glands. The calorigenic response in each case was proportional to the total iodine content. Diiodotyrosin peptone, obtained by peptic digestion of thyroglobulin, was inactive in one patient, whereas thyroxin peptone obtained in this way was active in the same patient in proportion to its iodine content

The results thus far indicate that no calorigenic activity is lost in the isolation of thyroglobulin from whole thyroid gland and that diiodotyrosin loses its activity in the first stage of proteolytic digestion of thyroglobulin

The Elastic Properties of the Emphysematous Lung and Their Clinical Significance By RONALD V. CHRISTIE, Montreal, Can

The elastic properties of the lung have been analysed in cases of emphysema by means of simultaneous tracings of the tidal air and interpleural pressure. With inspiration and expiration the interpleural pressure is found to fluctuate around that of the atmosphere, and an almost complete loss of the pulmonary elasticity can be demonstrated. These changes can also be shown by vital capacity tracings, the reserve air if taken alone being greater than that taken after the complementary air. The respiratory level also shows characteristic fluctuation.

The significance of these findings with regard to the increase in lung volume, and the dyspnoea and orthopnoea in emphysema is discussed, the paradoxical movement of the diaphragm during expiration being emphasized and explained on what is shown to be an active expiratory effort of the extrinsic muscles of respiration. To prevent this paradoxical movement a tight abdominal binder has been used in the treatment of emphysema, with encouraging signs of symptomatic relief.

The relationship of the loss of elasticity to unequal ventilation of the lungs in emphysema is also discussed.

The Association of Acalcification of Dentine with Hypoparathyroidism in Rats and the Cure of Same with Parathormone, with some Correlated Observations in Man By FULLER ALBRIGHT and (by invitation) MOSES S. STROCK, Boston, Mass

The first interrelationship to be demonstrated between the parathyroid glands and calcium metabolism was in 1911 by Erdheim. He showed that parathyroidectomy in the rat is followed by acalcification of that dentine which is formed subsequent to the operation. It has surprised some observers that acalcification should occur in hypoparathyroidism when decalcification is such an important part of hyperparathyroidism. The work of Erdheim has been repeated with similar results, and, with the aid of parathormone, strata of acalcified dentine followed by strata of calcified dentine have been produced at will. Decalcification of the dentine was never produced. The data are in accordance with the view that metabolic processes can cause acalcification of dentine, but not decalcification. Confirmatory observations from the teeth of patients with hypo- and hyperparathyroidism are made.

Observations upon the Refractory Period of the Auricular Extrasystole in the Mammalian Heart By E COWLES ANDRUS and (by invitation) PAUL PADGET, Baltimore, Md

The Production of Inflammatory Lesions in the Hearts of Animals Allergic to Hemolytic Streptococcus By CAROLINE C BEDELL and RAWLEY M PENICK (by invitation) and BENJAMIN M BAKER, JR, Baltimore, Md

Seegal, Seegal and Jost recently produced, by the intrapericardial injection of the homologous antigen an intense inflammatory reaction in the pericardium, heart and aorta of rabbits sensitized to egg white. The present report has to do with similar observations upon animals made sensitive to a beta hemolytic streptococcus. Twenty eight normal rabbits received, over a period of several weeks, 0.1 cc of a saline suspension of living organisms into the skin. At varying intervals after the beginning of sensitization the pericardial sacs were exposed and 2 cc of vaccine prepared from the streptococcus employed were injected. The animals were killed twenty-four hours to six days later and the hearts were examined both grossly and microscopically. Twenty five of these showed varying degrees of pericardial and myocardial inflammatory reactions somewhat similar to the changes described by Seegal, Seegal and Jost. One other had extensive changes confined to the pericardium only and the other two had but slight pericardial changes. Experimental lesions were compared with the spontaneous lesions of thirty one normal rabbits. Another control group was composed of twenty normal unsensitized rabbits which were subjected to the pericardial injections similar to those described above. Many of these had minimal pericardial inflammatory reactions but in all but one the heart muscles were normal.

The Action of Oxygen in Counteracting the Effects of Alcohol By ALVAN L BARACH, New York City, N Y

The ingestion of alcohol by a subject performing a standard exercise test resulted in a marked increase in the pulse rate, respiratory rate and pulmonary ventilation above the response of the same subject without alcohol. When oxygen was inhaled instead of air, there was a consistent reduction in the pulse rate, respiratory rate and pulmonary ventilation of the alcoholic subject. The inhalation of oxygen in the normal exercising individual also resulted in a lower pulse rate, respiratory rate and pulmonary ventilation than that obtained when the exercise was conducted in air, but the percentage difference between the effect of oxygen in the alcoholic subject was considerably greater than in the non alcoholic subject, suggesting that the inhalation of oxygen tended specifically to counteract the handicapping influence of alcohol on muscular exercise.

The psychological manifestations of alcoholism were slightly too moderately relieved or prevented by the inhalation of oxygen, but this varied considerably in individual subjects.

The Simple Dialysate Nature of Edema Fluids Contrasted with the Specialized Composition of Cerebrospinal Fluid By DOROTHY ROURKE GILLIGAN and MARIE C VOLK (by invitation) and HERRMAN L BLUMGART Boston Mass

The concentrations of certain electrolytes and non electrolytes in ascitic chest and subcutaneous edema fluid have been compared to the concentrations in both arterial and venous blood serum in twenty seven instances in patients with nephritis, cardiovascular disease, carcinoma and cirrhosis of the liver.

The differences in the concentrations of the various electrolytes between the sera and fluids are governed by the differences in the protein contents, regard

less of the site of fluid formation or the underlying diseased state studied. The distribution, in terms of water content, of sodium, chloride, and bicarbonate between serum and the edema fluids is in close agreement with that expected for simple dialysates in accord with Donnan's membrane equilibrium law, when the source of the blood sample is considered. The average distribution ratios found were

$$\frac{(\text{Na}^+) \text{ edema fluids}}{(\text{Na}^+) \text{ serum}} = 0.96, \quad \frac{(\text{Cl}^-) \text{ serum}}{(\text{Cl}^-) \text{ edema fluids}} = 0.98, \text{ and}$$

$$\frac{(\text{HCO}_3^-) \text{ serum}}{(\text{HCO}_3^-) \text{ edema fluids}} = 0.96,$$

for serum representative of blood midway between arterial and venous. The theoretic Donnan ratio for this series was 0.955. These results indicate that no bicarbonate is "bound" in the serum. The distributions of calcium, potassium, and inorganic phosphate indicate that fractions of these substances are bound in the serum. The concentrations of the non-electrolytes, sugar, total nonprotein nitrogen and creatinin were practically equal in serum water and corresponding fluid water, in accord with a simple dialysate nature for these body fluids. The distribution of the substances between serum and the edema fluids corresponds to the distribution found by Greene et al. (J Biol Chem, 1931, xci, 183) and others, between plasma and "in vivo" dialysates.

Our investigations show that the edema fluids studied are simple dialysates in equilibrium with blood plasma. This finding supports the hypothesis that these fluids have collected in abnormal amounts by virtue of an imbalance of the normal forces of formation and reabsorption and is in harmony with the concept that edema is primarily a quantitative rather than a qualitative abnormality.

A comparison of our studies with the spinal fluid measurements of other investigators has an important bearing on the theories regarding the mechanism of spinal fluid formation by the choroid plexuses, for it indicates that spinal fluid is not a simple dialysate. The concentrations, expressed in terms of water content, of sugar, creatinin, total nonprotein nitrogen, and inorganic phosphate, are much lower in the spinal fluid than in the serum, contrasted with practically equal concentration of these constituents in edema fluids and serum. The calcium content of spinal fluid is lower than the calcium content of edema fluid with similarly low protein content or than the "in vivo" dialysate. The difference in chloride concentration between serum and spinal fluid is much greater than the chloride difference between serum and the edema fluids or the "in vivo" dialysate, when the differences in protein concentrations between the sera and the two types of fluid are similar. Expressed in terms of water content, the ratio $((\text{Cl}^-) \text{ serum}/(\text{Cl}^-) \text{ spinal fluid})$ is too low to accord with the theoretic Donnan ratio and conversely, the ratio $((\text{HCO}_3^-) \text{ serum}/(\text{HCO}_3^-) \text{ spinal fluid})$ is too high. The choroid plexus must be considered a specialized structure and the fluid elaborated from it a specialized fluid rather than a simple dialysate.

The Basal Respiratory Quotient and the Effect of a High Fat Meal on the Respiratory Quotient and Heat Production of Normal and Obese Individuals. By BYRON D. BOWEN and (by invitation) FRED R. GRIFFITH, JR., and GRACE E. SLY, Buffalo, N. Y.

An attempt to demonstrate a qualitative metabolic difference between obese and normal individuals was undertaken. All subjects had previously taken an

unrestricted diet and were given the same test meal (100 grams of butter and 50 grams of mayonnaise) The Tissot spirometer and the Haldane gas analyzer were used Tests not showing fairly steady ventilation volumes and CO_2 percentages in the expired air were rejected in order to eliminate the influence of hyperventilation The effect of the meal was observed for six to ten hours

The basal respiratory quotients of normal and obese subjects were 0.825 and 0.767—an average of 13 and 21 determinations (subjects) respectively A similar difference was observed by Hagedorn et al, although the values obtained by them were higher due to a diet which had been rich in carbohydrate The ingestion of the fat meal brought the quotients of both groups to approximately the same levels—about 0.80, indicating that both groups derived their extra energy for the specific dynamic action from the combustion of a similar mixture of carbohydrate and fat This is opposed to the conclusions reached by Strouse and his coworkers from similar studies on a much smaller group

Similar studies made on a group of 11 obese diabetic patients at a time when the diabetes was under control by diet (but one had been taking insulin), showed the average respiratory quotient to be 0.765 The rise after the fat meal was less (0.782) than in the non diabetic obese group suggesting that the available carbohydrate reserve is less in the diabetic

The specific dynamic action was essentially the same for all groups, which opposes the idea that obese individuals are unique in this respect

Levels of Lactic Acid in Blood from Peripheral Vessels of Normal Subjects and Subjects with Claudication By GEORGE E BROWN and (by invitation) GRACE M ROTH, Rochester Minn

Levels of lactic acid sugar, oxygen content and saturation of the blood of the femoral and brachial arteries and femoral and basilic veins have been studied in normal subjects and in subjects with occlusive disease of the major arteries of the upper and lower extremities The relationship of levels of lactic acid and sugar has been investigated in relation to exercise and ischemic pains (claudication) The effect of cervicodorsal and lumbar sympathetic ganglionectomy on the lactic acid has been studied A correlation has been demonstrated between the levels of lactic acid in the venous blood and the excessive fatigue and pain of intermittent claudication The correlation is not sufficiently high however, to indicate a causal relationship between the two

Observations on the Circulation During and After Pregnancy By C SIDNEY BURWELL Nashville Tenn, and (by invitation) W DAVID STRAYHORN JR New York City N Y

Normal young women in the early months of pregnancy were selected and trained as subjects The acetylene method of Grollman was used to determine the cardiac output per minute it was applied under the usual standard conditions in regard to rest and food Observations were begun in the third or fourth month of pregnancy and continued at intervals of 2 to 6 weeks until delivery They were repeated during the puerperium in each case

In three of five women not only was there definite increase in cardiac output during pregnancy but this was greater than the increase in oxygen consumption so that the arteriovenous difference was decreased

In two patients there was no consistent elevation of cardiac output during pregnancy although isolated observations showed values above normal These patients had no unusual respiratory or circulatory symptoms or signs

In three women the cardiac output during the last weeks of pregnancy was lower than during several months preceding. In two of them it approached the values observed after delivery.

In all five women the cardiac output and the A-V difference after delivery were not beyond the limits of normal.

No striking variations in blood pressure were observed. The pulse was more rapid during than after pregnancy.

Water and Sodium Chloride Balance in Patients Before and After Surgical Operations By JOHN D STEWART and JOHN H TALBOTT (by invitation) and EDWARD D CHURCHILL, Boston, Mass.

The water and salt balances of 16 patients were studied in several types of surgical operations. In 3 patients on whom a herniotomy was done no significant negative balance of sodium chloride was observed. The largest decrease in serum chloride was 3.0 mEq, the average weight loss in the 5-day period of observation was 2.5 kgm.

In 4 patients on whom a partial gastrectomy was performed the average negative balance of sodium chloride was 44 grams. The average decrease in serum chloride was 2.7 mEq and the greatest weight loss was 1.7 kgm. In calculating the salt balance account was taken of chloride in vomitus and urine only. In the absence of diarrhea a small amount was presumed to be excreted in the stools. The remainder of the amount not accounted for was assumed to be lost in the sweat and drainage from the wound.

In 2 patients, on whom three-stage thoracoplasties were done, interesting findings were the changes in serum chloride. Four days after the first stage operation there was a decrease of 12.4 and 18.7 mEq in the respective patients. Following the second stage operation the greatest fall was 6.6 and 8.0 mEq and following the third stage was 1.4 mEq in the first patient. In the absence of noteworthy changes in the sodium chloride intake and output the above is taken as evidence of adjustment and adaptation to repeated loss of salt.

Transformation and Dissociation of Pneumococcus By M H DAWSON, New York City, N Y.

Previous work on transformation of type-specific pneumococcus by *in vitro* procedures is briefly reviewed. Evidence is presented which suggests that S forms of one specific type may be directly transformed into S forms of other specific types without passing through the intermediate R phase.

A new colony variant of pneumococcus is described. This new colony variant presents characteristics strikingly different from those of the previously described R and S forms. The morphology of the individual organisms constituting the new colony variant is also quite unique. Evidence is presented which suggests that the new phase of pneumococcus is an indirect and not a direct product of the parent form, from which it is derived. On the other hand reversion of the new phase to its parent form appears to be sudden and abrupt. The observations indicate that the evolution of the various phases may be cyclical in character.

Bacterial dissociation in other species will be briefly discussed and the place of the new phase of pneumococcus in the general phenomenon of bacterial dissociation indicated. The evidence suggests that it will be necessary to revise the currently accepted terminology for dissociate forms of pneumococcus in accord with the following: (1) M (mucoid)—formerly S (smooth), (2) S (smooth)—formerly R (rough), (3) R (rough)—not previously described.

Studies on Effective Barium Chloride Dosage By ARTHUR C DE GRAFF and
(by invitation) JEAN A CURRAN New York City N Y

In 1909 Rothberger and Winterberg's fundamental researches established the basis for the present clinical use of barium chloride. It was found that small amounts injected intravenously in dogs prevented ventricular standstill after combined nerve stimulation. Applying this finding to Adams Stokes' syndrome Cohn and Levine in 1925 prevented seizures of ventricular standstill with doses of 30 to 60 mgm three times a day. On the other hand as much as 0.6 of a gram a day has been given without toxicity or benefit. Because of this confusion as to dosage both therapeutic and toxic, the present investigation was undertaken first to ascertain if a definite end point of effective dosage could be determined second to determine the effective barium chloride dosage on dogs uninfluenced by any anesthetic, narcotic or operative preparation and third to compare dosage by vein and by mouth in both dog and man. Since in any drug in which the end point of therapeutic effect is in doubt, it is desirable to increase the dosage to the point of mild toxicity, experiments were carried through to this level.

It was found that in dogs 0.41 to 0.91 mgm injected intravenously per kilogram of body weight caused mild toxicity and unmistakable changes in the electrocardiogram namely ventricular premature contractions, frequently with coupling and changes in the character of the T wave. When given by mouth 97 to 189 mgm were needed to achieve similar results. Doses of over twice this amount caused moderately severe toxicity, but there were no fatalities. In man 0.63 to 0.88 mgm per kilogram of body weight were needed intravenously to give a definite end point, while 37 to 132 mgm per kilogram had to be administered by mouth to get parallel results. It was found that intravenously the greater the speed of injection the greater the effect. Electrocardiographic changes caused by barium chloride given by mouth generally persisted as long as 6 to 7 hours but disappeared inside of 18 hours.

Conclusions

1 The electrocardiogram reveals changes following barium chloride which are constant and furnish an end point as a definite guide in effective dosage. This is probably above the therapeutic dosage and though mildly toxic is far below the lethal dose.

2 The electrocardiogram furnishes a guide as to the rate of elimination.

3 Using these end points fairly constant relationship exists between the kilogram body weight and effective intravenous dosage.

4 For a given speed of injection there is a remarkably close comparison of effective dose by vein per kilogram of body weight between subjects both dog and man.

5 Using electrocardiographic changes as our end point of dosage we have shown the variability of comparative effective dosage by mouth from one subject to another.

An Aid in the Diagnosis of Subacute Bacterial Endocarditis By CLIFFORD
L DERICK and SAMUEL A LEVINE Boston Mass

Derick and Fulton in their paper on skin reactions of patients and normal individuals to protein extracts of streptococci reviewed the literature concerning the response of patients with subacute bacterial endocarditis to skin injections of streptococci and their products.

Since that time we have continued these observations. In all, 20 patients

with this disease have been studied. Thirteen of these showed a positive blood culture. The other 7 had all the clinical evidences necessary for this diagnosis.

Nucleoprotein extracts of hemolytic, green and indifferent streptococci were used as test materials. Only one case in which the diagnosis was not confirmed by blood culture showed a mildly positive response to the hemolytic streptococcus nucleoprotein. In all the other instances the tests were negative. In addition, one patient was tested with 0.2 cc. of a 24-hour broth culture of the autogenous viridans strain and another with approximately twenty million living organisms of *Streptococcus scarlatinae*. Both of these patients failed to show any reaction.

All cases where subacute bacterial endocarditis was suspected on admission were tested as a routine procedure. Since none of the patients proven to have subacute bacterial endocarditis showed a reaction, the finding of a positive test practically rules out this diagnosis. We have found this test of considerable diagnostic value.

The Effect of Arteriosclerosis and of Benign and Malignant Hypertension on the Area of Histamine Flares By A. CARLTON ERNSTE and (by invitation) MAURICE SNYDER, Cleveland, Ohio

In 30 normal individuals between the ages of 18 and 58 years, the average area of the flare produced by injection of histamine into the skin of the forearm was 31 sq. cm. The smallest flare observed had an area of 18 sq. cm., while an area of less than 25 sq. cm. was observed in only 6 individuals. In a group of elderly subjects with advanced grades of arteriosclerosis but with normal arterial blood pressure, flares of reduced area usually were recorded, while in other patients with slight or moderate thickening of the arterial walls, little or no diminution was the rule. Flares of normal area were obtained in 21 individuals with essential hypertension of the benign type. In 11 subjects with malignant hypertension, however, the average area of the flare was 14 sq. cm. and in only one patient did it exceed 19 sq. cm. Only two of the patients presented an advanced grade of arteriosclerosis.

Lewis demonstrated that the flare following a histamine stimulus results from dilatation of the strong arterioles of the skin. The diminution in the area of the flares observed in subjects with malignant hypertension and in those with advanced arteriosclerosis suggests the presence of structural changes in the walls of these vessels, although in malignant hypertension the possibility of severe and permanent vasoconstriction must be considered.

The results of the investigation indicate that measurement of the size of the histamine flare affords data of considerable importance in the clinical study of patients with essential hypertension.

The Relationship of Cerebrospinal Fluid Pressure to Systemic Blood Pressure By FRANK FREMONT-SMITH and (by invitation) H. HOUSTON MERRITT, Boston, Mass.

A comparative study of systemic blood pressure and cerebrospinal fluid pressure has been made in over 1600 patients.

In 1418 patients the systolic blood pressures ranged from 50 to 300 mm. Hg. and the diastolic blood pressures ranged from 30 to 190 mm. Hg. A study of these cases showed no significant correlation between the level of blood pressure and the cerebrospinal fluid pressure.

In 125 patients with elevated cerebrospinal fluid pressure, varying from 200 to over 1300 mm. of water, the blood pressure was rarely elevated excepting when the cerebrospinal fluid pressure was extremely high, approaching the level of the normal diastolic blood pressure. In 22 cases with cerebrospinal

fluid pressure varying from 500 to 800 mm of water, only 4 had a systolic blood pressure of 150 mm Hg or over

In 63 patients with uremia or congestive heart failure, the cerebrospinal fluid pressure was frequently elevated but showed no correlation with the systemic blood pressure

These results indicate that in man, as has been previously shown in animals, the cerebrospinal fluid pressure is not directly related to the systemic blood pressure and that an elevation of the cerebrospinal fluid pressure has no appreciable effect on the systemic blood pressure until the cerebrospinal fluid pressure level approaches the normal level of the diastolic blood pressure. When this occurs the systemic blood pressure becomes elevated to maintain cerebral circulation. A short discussion of the physiological mechanisms involved is given

Conclusions

- 1 The level of systolic or diastolic blood pressure has no appreciable influence upon the cerebrospinal fluid pressure

- 2 In uremia and congestive heart failure the cerebrospinal fluid pressure is frequently elevated

- 3 The level of cerebrospinal fluid pressure has no appreciable effect on systemic blood pressure unless the cerebrospinal fluid pressure is greatly increased so as to approach the level of the diastolic blood pressure. Under these circumstances there is a rise in the systemic blood pressure

The Interpretation of the Blood Sedimentation Rate (With Reference to Curves Obtained with Special Recording Apparatus) By A. PROSKOURIAKOFF (by invitation) and BURGESS GORDON, Philadelphia, Pa

Comparisons are made between curves obtained with the usual technique of interval charting and those with a special electrical recorder which plots continuously the level of sedimentation. The data suggest that variations in the degree and rate at certain periods may be significant in the interpretation of the curves

A Critical Examination of Methods for Determining the Cardiac Output in Patients with Cardiac Disease By T. R. HARRISON and (by invitation) ARTHUR GROLLMAN, BEN FRIEDMAN, and GURNEY CLARK, Nashville, Tenn

The accuracy and applicability of the acetylene method, the Burwell Robinson method and the venous plateau method have been studied by comparing them with each other and by testing the validity of the assumptions underlying them. In normal subjects and in certain persons with mild congestive heart failure the two latter methods agree excellently with the acetylene method which is based on an entirely different principle. However the three methods as ordinarily applied disagree in certain patients with heart failure and these have been the object of special studies from which the following conclusions are drawn

- 1 In certain cases with heart failure the Burwell Robinson method may occasionally yield inaccurate results and therefore cannot be used with safety

- 2 The venous plateau method gives accurate results when certain rigid criteria are adopted. For technical reasons and because of the discomfort to the subject involved in the procedure it is not usually the method of choice

- 3 The acetylene method may yield false results when only one determination of the arteriovenous difference is made at a single rebreathing. When two

measurements are made of the arteriovenous difference during a single rebreathing and are found to agree, the results are accurate. Because of its technical advantages this acetylene plateau method is the procedure of choice.

Criteria have been described whereby in a given case the applicability of a given method may be tested, so as to have no doubt of a given result. Unless these criteria are rigidly adhered to, markedly erroneous results may be obtained. In patients with severe congestive heart failure these criteria may be impossible of fulfillment.

The Interference with the Pulmonary Capillary Circulation by Fat and the Effect of Intravenous Alcohol Dextrose By LOUIS G. HERRMANN (by invitation) and GEORGE R. HERRMANN, Galveston, Texas

Rabbits and dogs were carefully selected and in double pairs injected at a uniform speed and without undue force at the rate of 1 cc per minute with warm (37° C.) liquid, sterile, neutral fat in doses of 1 cc per kilo of body weight. One animal of each series was kept as a control. Manifestations of disturbances of pulmonary circulation and the resulting anoxemia, as extreme restlessness, severe dyspnea and cyanosis, appeared rather promptly. The symptoms consistently disappeared almost immediately following the intravenous injection of 5 cc per kilo of body weight of the alcohol-dextrose solution. This was repeated at the end of 12 hours in two of the series and in 24 hours in one of the remaining two. At the end of 48 hours all animals were sacrificed and the lungs promptly and completely fixed showed a sharp decrease in the amount of neutral fat globules in the pulmonary capillaries according to the number of injections with almost a complete clearing in those animals that had received three injections.

Data are being accumulated on the types and amounts of neutral fat, fatty acids, cholesterol and phosphatids by volumetric determination in the arterial and venous blood, in the general and in the pulmonary circulation, before and after the injection of neutral fat, and after the injections of alcohol-dextrose. The quantitative distribution and the rate of disappearance of fat from the blood stream have been determined.

Clinical Manifestations of Hypo- and Hyper-Magnesaemia By ARTHUR D. HIRSCHFELDER, Minneapolis, Minn.

Although blood magnesium is usually constant (2 to 3 mgm Mg per 100 cc) in normal individuals, we have observed two distinct clinical groups in which significant alterations of blood magnesium were associated with concomitant clinical manifestations.

1 Low blood magnesium (0.9 to 1.37 mgm) was found in 10 cases, all manifesting twitching or convulsions. One of these was parathyroid tetany, two epilepsy, one cerebral injury, the others acute or chronic nephritis. Three of the latter were given Epsom salts by mouth, whereupon the blood magnesium rose to from 3.3 to 5.8 mgm Mg, and twitchings or convulsions disappeared.

There thus seems to be a definite clinical syndrome associated with low blood magnesium.

2 High blood magnesium. This was found especially in nephritics after the administration of Epsom salts. In normal individuals 20 to 30 grams $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ by mouth does not significantly raise the blood magnesium and about 40 per cent is excreted in the urine in 24 hours. In nephritics less of the ingested Mg is excreted in the urine, and the blood magnesium rises.

Twenty to 30 grams $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ administered to 9 patients with acute

and chronic glomerulonephritis raised blood magnesium from near normal to 98 to 113 mgm. All these patients were definitely more drowsy when at these levels. Two nephritics with blood magnesium 89 to 90 mgm without administration of $MgSO_4$ were definitely drowsy and unresponsive.

Coma is induced in animals when the blood magnesium approaches 17 mgm Mg per 100 cc.

Elevation of blood magnesium to about two thirds of the coma level thus seems to be accompanied by a tendency to somnolence.

It is probable that repeated purgative doses of Epsom salts by mouth can induce coma in nephritics, and that many cases of supposed uremic coma are really magnesium coma induced by Epsom salts. From this coma animals and probably patients can be awakened and their lives prolonged by intravenous calcium chloride.

Nephritic rabbits whose blood magnesium is moderately elevated (5 to 10 mgm Mg) by orally administered $MgSO_4$ are hypersensitive to morphine.

The Blood Pressure in Stenosis of the Isthmus (Coarctation) of the Aorta
By JOHN T. KING, Baltimore, Md.

The blood pressure in the arms in cases of isthmus stenosis (coarctation) of the aorta is so often greater than normal that many observers have assumed hypertension to be a necessary part of the diagnostic picture. In the author's experience hypertension was found in the arms in ten of eleven cases. One patient showed typical signs of coarctation including relative hypotension in the legs, but in her case the arm pressures were always within normal limits.

An analysis of the literature showed normal arm pressures present in twenty per cent of cases of isthmus stenosis in which pressure readings were recorded. The author's case is the seventeenth reported instance of normal arm pressure in coarctation of the aorta.

The Effects of Alternate Suction and Pressure on Blood Flow in the Lower Extremities of Normal Subjects and of Patients with Peripheral Vascular Disease
By EUGENE M. LANDIS and (by invitation) JOHN H. GIBSON, JR., Philadelphia, Pa.

The lower extremities of normal subjects and of patients with peripheral vascular disease were inserted into an aluminium box through a well fitted, heavy rubber diaphragm. Negative pressure, amounting to 120 mm Hg, was applied for 25 seconds alternating with positive pressure amounting to 100 mm Hg for 5 seconds.

Increase in blood flow to the distal portion of the lower extremity was identified by thermo electric measurements of digital skin temperatures. In normal subjects the extremity exposed to alternate suction and pressure cooled more slowly than the control extremity. When vasoconstrictor tone was diminished slightly by immersing one forearm in warm water the temperature of the extremity exposed to suction and pressure rose earlier, and to a greater height than that of the control extremity. With the subject chilly and the extremities cold suction and pressure in some observations had no measurable effect on blood flow until vasoconstrictor tone was slightly diminished by warming the body. In most instances the control extremity cooled while the rising temperature in the extremity exposed to suction and pressure indicated an increase in blood flow on that side.

In patients with peripheral vascular disease alternate suction and pressure was accompanied by conspicuous warming (and therefore by increased blood

flow) in extremities showing no rise in temperature during anesthesia of the posterior tibial nerve. In the presence of obstructive lesions of more moderate grade the more involved (and cooler) extremity was made the warmer of the two extremities by alternate suction and pressure.

The results indicate that this apparatus may possibly be used as a "peripheral heart" to increase blood flow in the lower extremities of patients with peripheral vascular disease.

The Normal White Blood Cell Picture By EDGAR JONES and DORAN J STEPHENS (by invitation) and JOHN S LAWRENCE, Rochester, N. Y.

Data have been collected from observations on sixteen normal adults which indicate that there are slight variations in the number of the white blood cells in the peripheral circulation under basal conditions. These fluctuations have been found to be greater than our error of technique. Determinations of the number of white blood cells have been made at 15 minute intervals over four hour periods. Differential counts have shown that all of the cells show fluctuations but the greatest of these are shown by the neutrophils. No rhythm, no showers of "non-motile" cells and no appreciable variations in the number of "stab" forms have been found.

The effect of change of posture has been carefully investigated in thirty-five normal adults and eighteen adults with a variety of clinical disorders. No significant variation has been found to be associated with change in position.

Observations on the effect of digestion on the white blood cell count in thirteen normal adults have shown a slight increase in the number of the cells in the majority of these individuals two to four hours after the ingestion of a large meal. The neutrophils account for most of the changes in the total number of white blood cells in these subjects.

Observations of the Cerebral Blood Flow in Man By F. A. GIBBS and L. L. GIBBS (by invitation) and W. G. LENNON, Boston, Mass.

A thermo-electric blood flow recorder has been devised for measuring the rate of blood flow through the internal jugular vein of man. Continuous records have been made over a period of three or four hours of the variations in flow through the internal jugular vein of unanesthetized human subjects. Records have been obtained of the flow associated with epileptic convulsions and with sleep. Observations so far fail to show significant changes in cerebral blood flow immediately preceding these events. The effect on cerebral blood flow of alterations in blood gases and of the administration of certain drugs has been studied. High CO_2 and low O_2 increase and low CO_2 decreases cerebral blood flow. Adrenalin produces a marked increase in flow, as do also amyl nitrite and acetyl cholin unless the fall in systemic blood pressure is too great. Caffeine and ergotamine tartrate produces a more lasting increase in cerebral blood flow than any of the other agents studied.

Single Cell Inoculations with Treponema Pallidum By CLARENCE S. THOMAS (by invitation) and HUGH J. MORGAN, Nashville, Tenn.

The development of syphilis in the rabbit following intratesticular injection of test material affords positive evidence of the presence of *Treponema pallidum* in the inoculum. Failure to thus infect has been interpreted as indicating the absence of *Treponema pallidum* in the inoculum. Inasmuch as this interpretation is widely used in the study of experimental syphilis (immunity, chemotherapy) it seemed desirable to test the validity of the assumption upon which it is based.

In preliminary experiments inocula, in which the presence of a few organisms seemed probable, failed to produce infection. An attempt was made to definitely determine this point by the inoculation of single organisms. A drop of diluted syphilitic testicular emulsion was placed in a moist chamber. By dark field illumination and with a micro manipulator it was possible to aspirate one or several organisms into fine glass pipettes. The pipette tips were immediately placed in syringe needles and injected into the testicles of rabbits. The testicles were then vigorously massaged.

Eighteen rabbits were thus injected with single organisms (11 Nichols strain 7 'S' strain). Four additional animals were inoculated, three with two organisms each (Nichols strain) and one with six organisms ('S' strain). All remained normal. At appropriate intervals transfers to a second series and from the second to a third series of rabbits were made all with negative results. Immunity to the homologous strain was not present in those animals of the third series which were re inoculated.

*The Cholesterol Content of the Blood in Obesity Including Observations of the Effect of a Diet Low in Fats Carbohydrates and Total Calories on the Blood Cholesterol*¹. By MAURICE BRUGER² (introduced by Herman O Mosenthal) New York City, N Y

Two hundred and seventy seven plasma cholesterol determinations were made on seventy five cases of obesity. The percentage of overweight varied from 20 to 122 per cent, with an average of 47.7 per cent for the group.

Uncomplicated (frank exogenous) obesity is associated with a normal plasma cholesterol. Diabetes mellitus, hypertension, arteriosclerosis and hypothyroidism tend to elevate the plasma cholesterol in the obese.

The normal plasma cholesterol in obesity is not affected materially by low-caloric, low fat diets in patients followed at regular intervals for approximately one year. The hypercholesterolemia in obesity complicated by diabetes mellitus, etc. usually shows a distinct elevation the first few weeks on low caloric, low-fat diets but tends to fall appreciably below the control level following several months on such a regime.

General considerations of diet and loss in weight in the obese in relation to the plasma cholesterol are discussed.

Leukocytosis Following the Intramuscular Injection of Liver Extract. By WILLIAM P MURPHY and (by invitation) JOHN H POWERS and KATHARINE HUMPHREYS Boston Mass., and Cooperstown N Y

Determinations of the total white cells and the total number and percentage of polymorphonuclear neutrophils in the peripheral circulating blood of 16 normal subjects before and after the intramuscular injection of liver extract have been presented in graphic form. The individuals were divided into 2 groups ambulatory and recumbent.

The average maximal increase in the total number of white cells of the ambulatory group was 94.1 per cent higher than the average of all control counts and occurred 7 hours after the extract was administered. The greatest individual response in this group was 185 per cent and the lowest was 23 per cent above the average of the 4 control counts made on each of these 2 subjects the previous day. The average maximal increase in total white cells of the patients of the recumbent group was 72 per cent above the normal level and

¹ Aided by a grant from the Josiah Macy Jr., Foundation
Oliver Rea Fellow in Medicine.

flow) in extremities showing no rise in temperature during anesthesia of the posterior tibial nerve. In the presence of obstructive lesions of more moderate grade the more involved (and cooler) extremity was made the warmer of the two extremities by alternate suction and pressure.

The results indicate that this apparatus may possibly be used as a "peripheral heart" to increase blood flow in the lower extremities of patients with peripheral vascular disease.

The Normal White Blood Cell Picture By EDGAR JONES and DORAN J STEPHENS (by invitation) and JOHN S LAWRENCE, Rochester, N Y

Data have been collected from observations on sixteen normal adults which indicate that there are slight variations in the number of the white blood cells in the peripheral circulation under basal conditions. These fluctuations have been found to be greater than our error of technique. Determinations of the number of white blood cells have been made at 15 minute intervals over four hour periods. Differential counts have shown that all of the cells show fluctuations but the greatest of these are shown by the neutrophils. No rhythm, no showers of "non-motile" cells and no appreciable variations in the number of "stab" forms have been found.

The effect of change of posture has been carefully investigated in thirty-five normal adults and eighteen adults with a variety of clinical disorders. No significant variation has been found to be associated with change in position.

Observations on the effect of digestion on the white blood cell count in thirteen normal adults have shown a slight increase in the number of the cells in the majority of these individuals two to four hours after the ingestion of a large meal. The neutrophils account for most of the changes in the total number of white blood cells in these subjects.

Observations of the Cerebral Blood Flow in Man By F A GIBBS and E L GIBBS (by invitation) and W G LENNON, Boston, Mass

A thermo-electric blood flow recorder has been devised for measuring the rate of blood flow through the internal jugular vein of man. Continuous records have been made over a period of three or four hours of the variations in flow through the internal jugular vein of unanesthetized human subjects. Records have been obtained of the flow associated with epileptic convulsions and with sleep. Observations so far fail to show significant changes in cerebral blood flow immediately preceding these events. The effect on cerebral blood flow of alterations in blood gases and of the administration of certain drugs has been studied. High CO_2 and low O_2 increase and low CO_2 decreases cerebral blood flow. Adrenalin produces a marked increase in flow, as do also amyl nitrite and acetyl cholin unless the fall in systemic blood pressure is too great. Caffeine and ergotamine tartrate produces a more lasting increase in cerebral blood flow than any of the other agents studied.

Single Cell Inoculations with Treponema Pallidum By CLARENCE S THOMAS (by invitation) and HUGH J MORGAN, Nashville, Tenn

The development of syphilis in the rabbit following intratesticular injection of test material affords positive evidence of the presence of *Treponema pallidum* in the inoculum. Failure to thus infect has been interpreted as indicating the absence of *Treponema pallidum* in the inoculum. Inasmuch as this interpretation is widely used in the study of experimental syphilis (immunity, chemotherapy) it seemed desirable to test the validity of the assumption upon which it is based.

water bath for 1 hour, the difference in O_2 content, minus that used by the blood alone, was taken as the amount consumed by the tissue. Liver and kidney tissues from 15 anemic and 15 control rabbits were used, the iron content of tissue and blood serum being determined.

Under these conditions tissues from anemic animals do not utilize as much oxygen as those of normal tissues by about 50 per cent. The results demonstrate the fact that, in general, anemia affects all the tissues and not the blood alone.

The Excretion of Bence Jones Protein in the Nephrotic Syndrome By HOWARD F. ROOT and (by invitation) HAZEL M. HUNT, Boston, Mass.

A case is presented of a 12 year old boy showing edema, albuminuria, low serum protein content, inversion of the albumin globulin ratio in blood serum and the hypercholesterolemia characteristic of nephrosis and excreting considerable amounts of albumin and of albumose of the Bence Jones type. The 24 hour excretion of albumin varied from a trace to 9.5 grams. The albumose varied in amount but it was possible once to isolate as much as 1.5 gram from a 24 hour specimen in a finely powdered condition by precipitation at $55^\circ C$. This isolated material gave the characteristic tests for Bence-Jones protein. The use of a high protein diet (protein 130 to 160 grams, carbohydrate 160 grams, and fat 100 to 150 grams) and thyroid extract (gr v daily) brought about the following results. In a period of 4 months anasarca disappeared, basal metabolism rose from -16 to $+7$ per cent, serum protein rose from 29 to 47 per cent. The albumin globulin ratio returned to normal. The excretion of Bence-Jones protein in the urine ceased. The plasma cholesterol rose steadily from 439 to 1250 mgm per 100 cc. A constant retention of nitrogen averaging 10 grams daily occurred during the first 6 weeks. Nitrogen balance was reached in 8 weeks. No cause other than the nephrotic syndrome was found for the occurrence of the Bence-Jones protein.

Even when the albumosuria was most marked no albumose was found in the blood plasma. This fact suggests that abnormality in the renal mechanism was responsible for the albumosuria rather than the formation of albumose in other tissues, transportation by the blood to the kidney and its excretion.

It has been held that the hypercholesterolemia of nephrosis is due to tissue starvation. The degree of tissue inanition in this case may be judged by the fact that for 6 weeks he retained nitrogen in amounts varying from 6 to 12 grams daily. The plasma cholesterol of 1250 mgm. is higher than any figure mentioned in Leiter's review of the subject. As yet the cholesterol has not begun to decrease although the clinical improvement is most marked.

The disappearance of edema when the serum protein remained at a low level indicates that some additional mechanism was involved in maintaining the osmotic pressure within the vessels. It is suggested that the high concentration of cholesterol acted in this manner to prevent edema.

Studies on Specificity of Streptococci. The Experimental Reproduction of Persistent Sneezing with a Streptococcus Isolated from a Case By EDWARD C. ROSENOW, Rochester, Minn.

The streptococcus isolated from the nasopharynx of the patient on the eighth day of persistent sneezing and from the brain and other tissues of inoculated animals was grown in dextrose brain broth and the cataphoretic velocity was determined in distilled water with the Northrop Kunitz Mudd apparatus.

Rabbits were inoculated intracerebrally, intratracheally and intraperitoneally, and dogs intracerebrally. Persistent sneezing, which continued to recur often

occurred 6 hours after the injection of liver extract. The highest and lowest individual responses in the members of this group were 101 and 20 per cent.

A similar increase in the total number of white cells and the total number and percentage of polymorphonuclear neutrophils was obtained in one patient with influenza.

The leukocytosis in every instance was due to an increase in the polymorphonuclear neutrophils.

The Occurrence of Pneumococcus Variants in Lobar Pneumonia By JOHN R. PAUL and (by invitation) J. A. LAWRENCE, New Haven, Conn.

Whether or not *S. pneumoniae* dissociate into avirulent variants during the clinical course of lobar pneumonia is the subject of this report. The work has been based upon recent observations upon the *in vitro* dissociation of *S. pneumoniae* which show that beside the well recognized and easily revertible, intermediate and R variants, there are also so-called R G variants, some of which are bile insoluble and apparently indistinguishable from *Streptococcus viridans*. These are difficult to revert back to the S forms.

Twenty-seven cases of lobar pneumonia were studied with daily throat cultures upon which differential bacterial colony counts were made. A shift in the throat flora was found at or about the time of crisis, S forms diminish, and suspected, unstable R and R G forms appear in great numbers during the first few postcritical days. To establish the identity of these suspected R G forms the attempt to revert strains isolated before and after crisis was made in about twenty-five instances. Three successful reversion experiments are reported upon R G strains isolated in the first week after crisis. The results suggest that virulent pneumococci undergo marked dissociation within the human host during pneumonia. The clinical implications are briefly discussed.

Observations on the Living Choroid Plexus By TRACY J. PUTNAM (Boston) and (by invitation) ERIC ASK-UPMARK (Lund, Sweden).

It is possible to observe the living choroid plexus of the cat under the microscope. By micrometry, the arteries can be seen to contract and expand under nervous and chemical influences, much as do the pial vessels. If the surface is stained with methylene blue, the choroid plexus, the muscular coat of the arteries and occasionally nerve fibers may be demonstrated. The axons of the subjacent white matter are easily stained. If fluorescein be injected intravenously, it may be seen to escape through the walls of the capillaries and veins, particularly the latter. This is perhaps of significance in relation to the pathogenesis of some cerebral diseases in which the lesions have a perivascular distribution.

Tissue Metabolism in Secondary Anemia By HERMAN H. RIECKER, Ann Arbor, Mich.

The object of this experiment was to determine whether the tissues of animals made anemic by hemorrhage could utilize oxygen at the same rate as those of normal animals. Previous work by Anson and Mirsky, Keilen, and Warburg indicated the possibilities that the iron-containing respiratory pigment (hem) of the cell might be partially depleted by iron starvation.

A simple method was devised which seemed suitable for the study. Normal defibrinated rabbit's blood was oxygenated and 1 to 2 grams of the tissue macerated and mixed with the blood. Twenty cc of the blood was used and the oxygen content determined before and after exposure to the tissue in a

water bath for 1 hour the difference in O content, minus that used by the blood alone, was taken as the amount consumed by the tissue. Liver and kidney tissues from 15 anemic and 15 control rabbits were used, the iron content of tissue and blood serum being determined.

Under these conditions tissues from anemic animals do not utilize as much oxygen as those of normal tissues by about 50 per cent. The results demonstrate the fact that, in general, anemia affects all the tissues and not the blood alone.

The Excretion of Bence Jones Protein in the Nephrotic Syndrome By HOWARD F. ROOT and (by invitation) HAZEL M. HUNT, Boston, Mass.

A case is presented of a 12 year old boy showing edema, albuminuria, low serum protein content, inversion of the albumin globulin ratio in blood serum and the hypercholesterolemia characteristic of nephrosis and excreting considerable amounts of albumin and of albumose of the Bence-Jones type. The 24-hour excretion of albumin varied from a trace to 9.5 grams. The albumose varied in amount but it was possible once to isolate as much as 1.5 gram from a 24 hour specimen in a finely powdered condition by precipitation at 55° C. This isolated material gave the characteristic tests for Bence Jones protein. The use of a high protein diet (protein 130 to 160 grams, carbohydrate 160 grams, and fat 100 to 150 grams) and thyroid extract (gr v daily) brought about the following results. In a period of 4 months anasarca disappeared, basal metabolism rose from -16 to +7 per cent, serum protein rose from 29 to 47 per cent. The albumin globulin ratio returned to normal. The excretion of Bence Jones protein in the urine ceased. The plasma cholesterol rose steadily from 439 to 1250 mgm. per 100 cc. A constant retention of nitrogen averaging 10 grams daily occurred during the first 6 weeks. Nitrogen balance was reached in 8 weeks. No cause other than the nephrotic syndrome was found for the occurrence of the Bence Jones protein.

Even when the albumosuria was most marked no albumose was found in the blood plasma. This fact suggests that abnormality in the renal mechanism was responsible for the albumosuria rather than the formation of albumose in other tissues, transportation by the blood to the kidney and its excretion.

It has been held that the hypercholesterolemia of nephrosis is due to tissue starvation. The degree of tissue inanition in this case may be judged by the fact that for 6 weeks he retained nitrogen in amounts varying from 6 to 12 grams daily. The plasma cholesterol of 1250 mgm. is higher than any figure mentioned in Leiter's review of the subject. As yet the cholesterol has not begun to decrease although the clinical improvement is most marked.

The disappearance of edema when the serum protein remained at a low level indicates that some additional mechanism was involved in maintaining the osmotic pressure within the vessels. It is suggested that the high concentration of cholesterol acted in this manner to prevent edema.

Studies on Specificity of Streptococci. The Experimental Reproduction of Persistent Sneezing with a Streptococcus Isolated from a Case By EDWARD C. ROSENOW, Rochester, Minn.

The streptococcus isolated from the nasopharynx of the patient on the eighth day of persistent sneezing and from the brain and other tissues of inoculated animals was grown in dextrose brain broth and the cataphoretic velocity was determined in distilled water with the Northrop Kunitz Mudd apparatus.

Rabbits were inoculated intracerebrally, intratracheally and intraperitoneally, and dogs intracerebrally. Persistent sneezing, which continued to recur often

for many hours, was produced by the different methods of injection of the live streptococcus as isolated and after many rapidly repeated subcultures in dextrose-brain broth, of suspensions of the heat-killed streptococcus and of filtrates of active cultures

The lesions produced were typical of encephalitis and were mainly in the medulla

The results in control animals injected in like manner with streptococci from patients having other diseases of the nervous system, were very different, in that sneezing was almost wholly absent and in that many animals revealed symptoms and other findings characteristic of the respective diseases studied

The cataphoretic velocity of the streptococci as isolated at the time of the attack or shortly after in the case of persistent sneezing, and in the control group, before and after animal passage, was mainly neurotropic. The neurotropic velocity and concomitantly the property of producing sneezing, were lost promptly on ordinary aerobic cultivation

A Significant Contrast Between Vasomotor Responses of the Peripheral Arteries By W J MERLE SCOTT and JOHN J MORTON, Rochester, N Y

To determine the respective contributions of vasoconstriction and of mechanical occlusion has become essential in the clinical analysis of arterial diseases in the extremities. For this purpose two different vasomotor responses of the peripheral circulation have been used, viz (1) the effect of vasoconstrictor paralysis and (2) that of vasodilator stimulation, the tacit assumption being made that the two resultant effects are approximately identical. In a group of fifteen individuals with normal blood vessels this is found usually to be the case, both paralysis of sympathetic vasoconstriction and stimulation of sympathetic vasodilatation causing an elevation of the surface temperature in the foot to the "normal vasodilatation level." Significant differences in the response of the local circulation to these two vasomotor reactions are found both in some individuals with normal and in those with pathological arteries. Occasionally the response to vasodilator stimulation is so much delayed or so much diminished as compared to the response to vasoconstrictor paralysis that the former is entirely unreliable for clinical purpose. As a corollary of this finding, in the clinical test unless an approximately maximal response to vasodilator stimulation is obtained the effect of vasoconstrictor paralysis should be ascertained.

On the Diagnosis of Myocardial Disease by Means of the Basal Cardiac Work

By ISAAC STARR, JR, and (by invitation) J S DONAL, L H COLLINS, JR, A MARGOLIES, and C J GAMBLE, Philadelphia, Pa

The normal standards for basal cardiac work (cardiac output multiplied by mean blood pressure) have been determined by Starr, Collins and Wood (J Clin Invest, 1933, 11, 13). Since that investigation the basal cardiac work and the heart size have been determined for about 100 additional cases, including all the types of heart disease that could be secured. In this presentation it is proposed to discuss these results from the clinical point of view.

All cases in, or obviously threatened with, congestive failure were found to have myocardial insufficiency, i.e. their hearts were doing abnormally small amounts of work per unit of heart size. Therefore these estimations may appear to be of value in the diagnosis of this condition. A considerable number of cases of hyperthyroidism and hypertension showed the same abnormality.

On the other hand the majority of cases of angina pectoris and coronary

occlusion (an old infarct was later demonstrated at autopsy in two patients) had a normal basal cardiac work

Cases of effort syndrome show a small basal cardiac work, but give no evidence of myocardial insufficiency because they have small hearts and the work per unit of heart size is within normal limits

Alterations in the Specific Gravity of the Plasma of the Blood with the Onset of Diuresis in Patients Suffering from Heart Failure of the Congestive Type By HAROLD J STEWART New York City, N Y

The mechanism of the occurrence and elimination of edema in heart disease is still incompletely understood. Where, in a system regarded as circulating blood located between the tissues and the kidneys, must a stimulus be applied in order that diuresis may be initiated? Different types of diuretics may obviously act at different points. If the kidneys are first affected so that fluid passes from the circulating blood *concentration* of the blood should occur. If, on the other hand, the process is initiated in the tissues, fluid first enters the circulating blood and *dilution* occurs and may be detected if it lasts long enough and is great enough.

Light on what the occurrences are when edema disappears may be gained by studying the blood to see whether concentration or dilution takes place. Changes in the specific gravity of the blood plasma may be utilized to trace the alterations in the blood. The method of Van Slyke and Moore has been used. It is now known that the specific gravity of the plasma of normal individuals varies from day to day within narrow limits only. It is moreover closely correlated with the amount of protein present in the blood. In cardiac patients the occurrence of edema does not depend on the level of specific gravity of the plasma which has in fact a normal value. We have investigated the curve of specific gravity with the onset and continuance of diuresis in patients suffering from heart failure of the congestive type who exhibited edema and accumulations of fluid. One case will serve to illustrate the alterations which were observed. B McL., female, white 56 years of age suffering from a second attack of heart failure of the congestive type, exhibited edema of the extremities. Arterial hypertension and enlargement of the heart were present. Rapid ventricular rate due to auricular fibrillation was present. Although the intake of fluids was restricted to 1200 cc. per day and she remained in bed the output of urine did not rise beyond 200 to 300 cc. per day and she did not lose weight. The specific gravity of the plasma of the blood measured 1.0278. Digitalis 12 gram was given within 24 hours, the ventricular rate became slower, diuresis occurred and the patient lost weight. The specific gravity of the plasma fell to 1.0240 and remained in that zone during diuresis but when diuresis stopped the specific gravity returned to the level it had had beforehand (1.0280). Diuresis sometimes was accompanied by similar changes when it occurred spontaneously as a result of limitation in the administration of fluids and of rest in bed and also as a result of giving theocalcin. By taking samples of blood frequently it was found that decrease in the specific gravity of plasma appeared to precede the onset of diuresis and that it was possible to predict the occurrence of diuresis. Estimation of the total amount of protein excreted by way of the urine during the periods of diuresis failed to account for the low concentration of protein indicated by the alteration in specific gravity. The decrease was due not to loss of protein from the blood, but to dilution of the blood by edema fluid. In another patient, in whom the specific gravity of the plasma of the blood before the onset of diuresis was 1.0270 the γ_{50} of the edema fluid was 1.0084. With the occurrence of γ_{50}

gravity of the plasma fell to 1.0240, had approximately 400 cc of edema fluid having a specific gravity of 1.0084 entered the blood, the amount would have been sufficient to reduce the specific gravity of the plasma to the observed level (1.0240)

Using alteration in specific gravity as a measure of concentration of protein in the blood, it appears that diuresis, in the presence of heart failure of the congestive type, depends on changes initiated in the tissues, and is accompanied by dilution of the circulating blood

Constitutional, Hereditary and Familial Features of Pernicious Anemia By CYRUS C. STURGIS and RAPHAEL ISAACS and (by invitation) L. R. GATES, Ann Arbor, Mich

A study of 680 consecutive patients with pernicious anemia and a similar number of non-pernicious anemia patients of the same locality and age groups was made, correlating the weight, hair color, eye color, ear length, blood pressure, spleen size, blood count, blood bilirubin, lymph node size, heart size, heart murmurs, gallbladder, age incidence, duration and individual symptoms. The disease incidence in the family and system incidence in the patients were correlated and the significance or non-significance was determined from Fisher's table of χ^2 . A similar study was made, correlating the various symptoms of the patients. Pernicious anemia occurred more frequently in the families of the pernicious anemia patients than in the others. No significant correlation was evident between the special system disease tendency in any individual patient and a history of similar system disease in the family, although there was a moderate tendency for the association of these symptoms in the presence of a family history of heart disease, gastro-intestinal, genito-urinary, and skin disease and tuberculosis.

The Absorption of Thyroxine from the Gastro-Intestinal Tract, with Special Reference to the Effect of Alkali By WILLARD O. THOMPSON and (by invitation) PHEBE K. THOMPSON, SAMUEL G. TAYLOR, III, and LOIS F. N. DICKIE, Chicago, Ill

A large number of observations on twelve patients with myxedema show that pure synthetic thyroxine when given by mouth or directly into the duodenum has only a slight effect on the basal metabolism, whereas, when given by mouth in the form of its monosodium salt, it has about one-quarter to one-fifth as much effect as when given intravenously, and when given in solution with an excess of sodium hydroxide, it has a much greater effect which nearly equals its effect by the intravenous route. Dissolving thyroxine in an excess of sodium hydroxide results in the formation of the disodium salt. Squibb's thyroxine for oral use, which presumably contains a sodium salt of thyroxine and some thyroxine in peptide combination, has about two-thirds to three-fifths as much effect as thyroxine intravenously and about three times as much effect as the monosodium salt of synthetic thyroxine given orally.

Since pure thyroxine is insoluble in most solvents and its monosodium salt only slightly soluble, while the disodium salt and thyroxine in peptide combination are much more soluble, it would appear that the solubility of the thyroxine compound administered is an important factor in its absorption from the gastro-intestinal tract and hence in its effect on the basal metabolism. The solubility of thyroxine peptide and polypeptide derived from desiccated thyroid during digestion in the gastro-intestinal tract may explain the fact that desiccated thyroid by mouth has about the same effect as thyroxine intravenously on the basis of equivalent iodine contents.

The Dissolution of Clotted Plasma by Hemolytic Streptococci: the Relation of This Phenomenon to Acute Hemolytic Streptococcus Infections By WILLIAM S. TILLET and (by invitation) R. L. GARNER, Baltimore, Md.

Hemolytic streptococci isolated from patients are capable of rapidly liquefying clotted human plasma. Sterile Berkefeld filtrates of full grown broth cultures contain the fibrinolytic property to a high degree. Broth cultures and sterile filtrates of hemolytic streptococci (beta type) derived from human cases possess this property when tested with clotted human plasma. The human strains fail completely to liquefy the fibrin clot of plasma derived from many animals. Available strains of hemolytic streptococci derived from animals are incapable of liquefying human clot. Fibrinogen, chemically isolated from plasma, when coagulated with thrombin in the presence of suitable culture or filtrate is rapidly liquefied. Dissolution under these conditions occurs with both animal and human material. The rate of liquefaction is such that 0.5 cc. of culture or filtrate transforms 1 cc. of solid clotted plasma into a completely fluid state within fifteen to twenty minutes. A comparable amount of fibrinogen, when coagulated in the presence of culture, is completely liquefied in five minutes.

Pathogenic bacteria, such as pneumococci, green streptococci, typhoid bacilli, etc., similarly tested, have not exhibited a comparable fibrinolytic property.

The fibrin digesting power of hemolytic streptococci suggests an explanation for the manifestations of acute streptococcus infections which are characterized by thin fibrin poor exudates such as are seen in the early stages of hemolytic streptococcus empyema.

By repeated tests on plasma obtained from patients ill with hemolytic streptococcus infections, recovery frequently parallels or is followed by the development in the blood of a property by which clot digestion is inhibited. Effective resistance to the activity of the fibrinolytic ferment is most commonly demonstrable in the plasma of patients recovered from suppurative hemolytic streptococcus infections. The development in the blood of recovered patients of the capacity to inhibit this bacterial activity seems to be a form of resistance which differs in the parallel tests so far performed from streptococcal agglutinins or the anti streptolysin described by Todd.

Observations on the Etiological Relationship of Severe Alcoholism to Pellagra

By TOM D. SPRES and H. F. DEWOLF (introduced by Joseph T. Wearn), Cleveland, Ohio

It is generally agreed that the pellagra found in the North is usually associated with severe alcoholism. This study demonstrates that over ninety per cent of the patients develop the lesions of pellagra following excessive drinking. Ten patients having typical "alcoholic pellagra" recovered from their disease while receiving an adequate diet and large quantities of corn whiskey. On theoretical grounds it may be considered that the whiskey predisposes to the development of pellagra either by destroying the so-called "pellagra preventive" factor or by altering the gastro-intestinal tract so that it becomes incapable of assimilating the preventive substance. Likewise it has been suggested on theoretical grounds that foreign substances in the whiskey such as the higher alcohols produce the lesions of pellagra.

It appears unlikely however that there is any specific deleterious material present in the many kinds of beverages taken. The present observations show that the consumption of large quantities of whiskey at the same time that an adequate diet is received does not interfere with the clinical improvement of

the patients. This is strong evidence that alcohol does not directly inactivate either all of the gastric secretion or all of the potent substances in the food. It is well known that many individuals drink large quantities of alcoholic beverages for years without suffering from loss of appetite, vomiting or pellagra. Correlation of these findings suggests the following hypothesis: Severe alcoholic imbibition by some individuals causes inadequate ingestion of food by decreasing the appetite and often precipitates nausea and vomiting. These predispose to the development of pellagra because the person no longer receives an adequate diet but utilizes the calories in the alcohol.

The Clinical Significance of the Hyperactive Carotid Sinus Reflex By SOMA WEISS and (by invitation) JAMES P. BAKER, Boston, Mass.

A study has been made of the circulatory and nervous system of 12 subjects who complained of dizziness and fainting, and in whom the mechanical stimulation of one or each of the carotid sinuses induced characteristic aura, dizziness, fainting and convulsive seizures. Three main types of cardiovascular responses were observed following stimulation of the sinus: (a) marked asystole or sudden slowing of the heart rate with or without marked fall in the arterial blood pressure, (b) marked fall in the arterial blood pressure, but without essential cardiac slowing, (c) changes in the cerebral circulation without essential slowing of the heart rate, and without fall in the arterial blood pressure. Stimulation of the hyperactive carotid sinus reflex induced striking changes in the intracardiac conductive system: partial and complete heart block, temporary asystole of the ventricle with continued auricular contraction, nodal rhythm, ventricular extrasystoles, change in the shape of the T waves, and complete inversion of the electrical axis in the heart. The observations also demonstrate that Adams-Stokes syndrome can be induced reflexly.

Evidence is presented that the clinical symptoms and signs observed, as well as the cardiovascular changes, are due to stimulation of the sinus and not to direct motor vagal stimulation.

During stimulation of the reflex, the volume and velocity of blood flow became decreased. There was also a slowing of the blood flow through the brain. In the precipitation of fainting and convulsions the rate (time element), rather than the absolute deviation from the normal circulatory state of the brain, plays the primary role. This temporary, sudden ischemia, even if of short duration, sets up a sequence of events in the brain which proceeds independently to convulsions even if hyperemia promptly follows the ischemia.

Section of the carotid sinus nerve in two cases abolished the spontaneous fainting attacks.

Studies on the Prevention of Cholesterol Atherosclerosis in Rabbits By KENNETH B. TURNER and GEORGE B. KHAYAT (introduced by Randolph West), New York City, N. Y.

Atherosclerosis of the aorta was readily produced in rabbits by feeding cholesterol. The lesions were prevented in 17 of 19 animals by the simultaneous administration of dried whole thyroid gland with the cholesterol, while comparable thyroxin dosage was effective in only 3 of 10 rabbits. The changes produced by cholesterol were also prevented by potassium iodide in 17 of 18 animals. The bromide and chloride of potassium were ineffective. If complete thyroidectomies were performed upon the rabbits, potassium iodide no longer succeeded in preventing the intimal changes in the aorta. A close parallelism was observed between the level of the blood cholesterol and the presence or absence of arterial lesions.

The Mitral Diastolic Murmur in Children without Mitral Valve Deformity but with Severe Rheumatic Carditis and Dilatation of the Left Ventricle By PAUL D WHITE and (by invitation) EDWARD F BLAND and T DUCKETT JONES, Boston, Mass

The Electrocardiogram in the Earlier Stages of Experimental Myocardial Infarction By FRANK N WILSON, PAUL S BARKER and (by invitation) FRANKLIN D JOHNSTON, IAN G W HILL, and GERALD C GROUT, Ann Arbor, Mich

A series of experiments was carried out in dogs in which various branches of the coronary arteries were ligated. Electrocardiographic studies were made either immediately after the ligation, or at some later period within the first twenty-four hours. In addition to the standard electrocardiographic leads and serial precordial leads, direct and semi direct leads from the exposed heart were employed.

Immediately after ligation direct leads from the region supplied by the tied vessel yielded nearly monophasic responses, similar in all respects to those obtained from a region of the ventricular surface recently injured by burning or by other means. The RS-T deviation shown by these monophasic curves rapidly diminishes but does not entirely disappear until the infarcted muscle dies some twelve or fifteen hours after ligation. The curious T wave changes that occur in the coronary occlusion in man are also seen in direct leads from the margin of the infarcted area some hours after ligation. They are apparently due to alterations in the recovery process in this region.

Hypertension with Arteriolar and Glomerular Changes in the Albino Rat Following Subtotal Nephrectomy By J EDWIN WOOD JR, and (by invitation) CLAYTON ETHRIDGE, University, Va

Hypertension and renal insufficiency following partial nephrectomy in rats have been reported by Chanutin and Ferris. It is the primary purpose of this study to describe the histological changes in the rat kidney subjected to functional strain by subtotal nephrectomy and to investigate a possible relationship between these changes and hypertension.

One hundred and eighty rats have been fed on a balanced diet. One hundred and fifty three of these have had 80 per cent of the kidney tissue removed and 27 control animals, simple laparotomy but no nephrectomy. Direct carotid blood pressure readings have been made and autopsy performed at intervals varying from 1 to 267 days. Of 153 animals with subtotal nephrectomy 68 have had blood pressures ranging from 70 to 139 mm Hg, 42 from 140 to 169 mm Hg, 32 from 170 to 199 mm Hg, and 11 from 200 to 230 mm Hg. The higher blood pressure levels have been found in rats with subtotal nephrectomy of 60 days duration or longer and in each instance of hypertension degenerative glomerular changes have been present. The blood pressure in 27 control rats varied between 100 and 140 mm Hg with an average of 120 mm. Hg, a figure already fully established by Chanutin and Ferris.

During the early days of the experiment the tubules show rapid dilatation and epithelial hyperplasia with marked diffuse fatty change. This tubular fatty change is transient but reappears in localized areas at a later stage. Early, the glomeruli are distended and enlarged but free of degeneration.

Definite glomerular involvement begins to appear at approximately 40 to 70 days after operation as shown by marked epithelial cell enlargement with subsequent vacuolar degeneration and glomerular adhesions to the capsule. In

the older animals there is further glomerular degeneration with a gradual loss of structural detail and progression to varying degrees of hyalinization and fibrosis. Glomerular fatty degeneration is striking and begins to appear in 80 to 120 days. This is also progressive and is marked after 170 days. Fatty degeneration of the afferent arterioles has been found at still later stages. These time intervals may vary. None of the pathologic changes has been seen in the control animals.

Summary Hypertension frequently follows subtotal nephrectomy in rats. Fatty degeneration in the glomeruli and afferent arterioles, glomerular atrophy and hyalinization and tubular dilatation following subtotal nephrectomy have been observed and may be interpreted as a natural sequence of hypertrophy and degeneration brought about by functional strain.

The Response with Respect to Serum Volume and Composition after the Ingestion of Glucose in Diabetic Patients By F. WILLIAM SUNDERMAN and (by invitation) ENNION WILLIAMS, Philadelphia, Pa.

Blood was removed from diabetic patients, fasting, and one and one-half hours after the ingestion of glucose. The increase in the glucose concentration in the serum per kilogram of water was found to be accompanied by a decrease in the chloride concentration in approximately the ratio of 3.8 mM to 1. If the assumption be made that the solids of the serum, excepting glucose and chloride, are constant and on this basis the amounts of glucose, water, and chloride per unit of the remaining solids are calculated before and after the administration of glucose, it is found that the increase in the glucose is accompanied by an increase in both water and chloride. The solution transferred to the serum with the glucose is a hypotonic saline solution of an average chloride concentration of 60 mM per kilogram of water, but with the addition of the glucose, it has on the average a higher osmolar concentration than the serum. The final result, therefore, is an increase in the glucose concentration in the serum, a decrease in the chloride concentration, an increase in the total osmotic pressure, and an increase in the serum volume. This would indicate that in the serum of the diabetic patient the disturbance resulting from the uptake of glucose is distributed among at least three other variables—serum volume, osmotic pressure and chloride concentration.

THE DIURNAL RHYTHM IN WATER AND MINERAL EXCHANGE¹

By ROBERT C MANCHESTER

(From the Department of Pediatrics The University of Rochester School of Medicine and Dentistry Rochester New York)

(Received for publication May 16 1933)

In the course of investigations on total mineral and water exchange in epileptic children (1), a diurnal rhythm in the excretion of water and certain minerals was regularly encountered, which persisted even under stringently abnormal metabolic conditions. Recently, these observations have been confirmed and further elaborated in a series of studies on two mild epileptic patients and one normal male subject.

It is common knowledge that urine is excreted in larger amounts during the day than at night, and that the increased urine volume of the day is associated with a negative water balance, counterbalanced at night by a positive balance due to a decrease in urine output. Associated with the period of retention, Simpson (2) found that an increase occurred in urinary hydrogen ion concentration, titratable acidity and ammonia, while chloride excretion fell below that of the day period.

Norn (3) gave subjects equal amounts of food and water at three hour intervals throughout the day and night. The excretion of sodium, potassium and chloride was found to be lower at night than during the day, reaching a minimum between 3:00 A.M. and 6:00 A.M. and a maximum between 12:00 noon and 3:00 P.M. The rhythm seemed to be dependent on the degree of activity. It was reversed when the subject slept through the day and worked through the night period.

Other papers have appeared in the literature dealing with the excretion of various urinary constituents over day and night periods but as far as the author is aware a composite study is not available which includes gross water exchange and the more important urinary elements.

PLAN OF STUDY AND METHODS

The greater part of the data to be presented has been selected from a series of investigations on two mildly epileptic male children carried out over a period of forty consecutive experimental days. No convulsions occurred throughout the entire study.

Except for changes induced every effort was made to maintain the environmental and metabolic conditions as constant as possible not only from day to

¹ This investigation was aided by a grant from the Research Fund of the Rockefeller Foundation.

day, but also for each period of the day. The subjects were kept in bed under sufficient bed covers to prevent chilling or sensible perspiration. Room temperature and relative humidity were recorded every two hours. Humidity ranged from 40 to 80 per cent and room temperature from 22° to 25° C, only occasionally rising high enough to induce sensible perspiration in the axilla. Activity was regulated and recorded by a trained nurse in constant attendance.

Each day was divided into four periods of six hours beginning at 6 00 A M. At the beginning of each period, the subject voided, was weighed on scales sensitive to five grams and was then given an accurately prepared meal of known weight and composition.

The four meals of the twenty-four hour period were identical in every respect and were prepared by a trained attendant in an adjoining kitchen from simple foods such as powdered milk,² 40 per cent cream, lactose, egg yolk, sodium chloride, and distilled water. A sufficient quantity of lactose and powdered milk was obtained to meet the needs of the entire study, thus reducing variations in the diet to a minimum. That metabolic and mineral requirements were adequately met is indicated by the uniformity in mineral and water excretion during control periods. The ketogenic antiketogenic ratio of the metabolic mixture was slightly below unity.

Water balances were calculated by the method suggested by Newburgh, Johnston, and Falcon-Lesses (4). The subjects were in nitrogen equilibrium and the caloric value of the food intake was approximately equivalent to the caloric requirement so that non-oxidation water freed by the catabolism of body tissue or stored with any protein or fat deposited in the body was disregarded. "Total water available" includes drinking water, preformed water of the foods, and water of oxidation of the metabolic mixture. "Total water loss" includes water of the urine, water of the feces, and water lost insensibly by way of the skin and respiratory tract.

The water content of the urine and foods was determined by weighing 5 to 15 gram samples before and after desiccation in vacuo over sulphuric acid. The nitrogen of the foods, urine, and feces was determined by the Kjeldahl method (5). Urinary chloride was determined by the Vohlhard method (5), inorganic phosphate by the method of Fiske and Subbarow (6), sulphate by the method of Fiske (7), titratable acidity by the method of Folin (8) and ammonia by the permutit method of Folin and Bell (9). For the other mineral analyses urines were first ashed by treating with small amounts of nitric, perchloric, and sulphuric acids. Using the ashed specimens, sodium was determined by the method of Barber and Kolthoff (10), calcium by the gasometric method of Van Slyke and Sendroy (11), potassium by the method of Shohl and Bennett (12), and magnesium by a method adapted by the author which was based upon the precipitation of magnesium as magnesium ammonium phosphate and the measurement of the phosphate radicle by the Fiske and Subbarow method (6). All samples of blood were drawn under oil without venous stasis.

Unless otherwise stated, the subject followed a definite routine of activity consisting of games, reading and regulated naps from 6 00 A M. to 6 00 P M., and slept from 6 00 P M. to 6 00 A M. except for interruptions resulting from the experimental routine. Since identical meals were taken at equal intervals throughout the day and night, all variables except activity and sleep and the resulting metabolic factors were reduced to a minimum.

² Klim

RESULTS OF EXPERIMENTS

Diurnal rhythm under standard metabolic conditions

The diurnal variations regularly encountered are clearly demonstrated in Table I. In Part I, a negative water balance is noted during the day period and a positive balance at night, dependent largely on the fluctuation in urine volume. Associated with the negative balance, urine volume, and urinary sodium, potassium, and chloride excretion reaches a maximum, while during the positive water balance of the night period, excretion falls to a minimum. A similar less significant rhythm occurs in titratable acidity, and phosphate, sulphate, and ammonia. "Total inorganic acid" and "total base" is greater during the day than at night. The difference is obviously largely dependent on the sodium, potassium and chloride fractions. Nitrogen excretion and body temperature are greater in the daytime than at night. Insensible perspiration shows no constant variation.

In a more detailed experiment presented in Part II in which the day is divided into six hour periods, the same diurnal rhythm is demonstrated. The negative water balance, urine volume, and sodium, potassium, and chloride excretion reach a maximum in the morning period from 6 00 A M to 12 00 noon and begin to decline in the afternoon. Excretion decreases sharply through the night and reaches a minimum in the period from 12 00 midnight to 6 00 A M coincident with the period of greatest water retention.

In Table II, the data of Table I, Part I, have been recalculated to show the variation in concentration of the more important urinary constituents in day and night specimens. The molar concentration of those substances showing only a slight diurnal rhythm, namely phosphate, sulphate, and ammonia increases slightly in the night urine. In the case of sodium, potassium, and chloride, the decrease in excretion during the night is parallel to a decrease in urine volume so that no regular change in the molar concentration of these elements occurs. Fluctuations in the total molar concentration of the inorganic ions are insignificant and the higher specific gravity of the night urine is due largely to an increase in the molar concentration of the nitrogenous substances, of which urea is the most important.

The marked diurnal fluctuations in urinary sodium, potassium, and chloride excretion suggest the possibility of a corresponding change in the quantity of these elements in the blood. Unfortunately, only one experiment bearing on this point was carried out.

The results, presented in Table III show no significant change in serum sodium or chloride in spite of a wide difference in urinary sodium and chloride excretion between the day and night period. Considered as a single experiment, no conclusions can be drawn. The results, however, are in accord with those of Norn (3).

TABLE II

Comparison of the molar concentration of urinary constituents in day and night periods Urea is calculated as 80 per cent of the urinary nitrogen
H D, age 5 years Weight 20 kgm

Time	Volume	Specific gravity	Inorganic acid				Inorganic base					NH ₃ concentration	Urea concentration	Total concentration
			Cl Concentration	P Concentration	S Concentration	Total Concentration	Na Concentration	K Concentration	Ca Concentration	Mg Concentration	Total Concentration			
	cc.		M per liter	M per liter	M per liter	M per liter	M per liter	M per liter	M per liter	M per liter	M per liter	M per liter	M per liter	
6 A M - 6 P M	523	1 015	035	007	002	044	047	034	001	004	086	028	199	357
6 P M - 6 A M	219	1 025	028	017	004	049	051	034	003	010	098	058	465	670
6 A M - 6 P M	621	1 010	028	006	002	036	033	029	001	003	066	024	168	294
6 P M - 6 A M	253	1 021	027	014	003	044	047	031	002	010	090	058	356	548

TABLE III

Comparison of urinary sodium and chloride excretion with the level of sodium and chloride in the blood serum in day and night periods R C M
age 25 years Weight 63 kgm

Activity	Time	Water balance			Urine					Blood serum		
		Total water available	Total water lost	Balance	Volume	Specific gravity	Na	K	Cl	Time	Na	Cl
Active Sleeping	6 A M - 6 P M	grams 1548	grams 1854	grams -306	cc 1111		mgm 1500	mgm 2033	mgm 3442	12 noon	mgm 321	mgm 354
	6 P M - 6 A M	grams 1548	grams 1237	grams +311	cc 723		mgm 1070	mgm 853	mgm 1476	12 midnight	mgm 326	mgm 350

The effect of activity on the diurnal rhythm was studied in a short experiment presented in Table IV. On the second day, the routine was altered so that the subject remained inactive, but awake, lying quietly on his back, from 6 00 A M to 12 00 noon. In spite of the reduced activity, the usual matutinal rise in urine volume, and sodium, potassium, and chloride excretion occurred. He was allowed to sleep as usual from 6 00 P M to 12 00 midnight but was kept awake and active from midnight to 6 00 A M. Coincident with the resumption of activity, water balance became negative, and urine volume, sodium, potassium, and chloride excretion increased. The subsequent sleeping period from 6 00 A M to 12 00 noon was unsatisfactory because of restlessness on the part of the subject. Urine and mineral excretion remained at about the same level as in the preceding period, but in spite of this they increased in the subsequent period in which normal activity was resumed.

Although the experiment is not entirely satisfactory, the results are in accord with those of Norn (3) who was able to reverse the rhythm in subjects working at night and sleeping by day.

The diurnal rhythm under abnormal metabolic conditions

A few experiments which were made under abnormal experimental conditions are presented in order to demonstrate the tenacity with which the rhythm is maintained even under extreme metabolic conditions.

The data presented in Part I of Table V are selected from a period in which ingestion of water was being forced. In spite of the large urine output, the volume of urine of the day exceeds that of the night period and the diurnal fluctuation in sodium and chloride excretion persists.

Even in a state of dehydration, as shown in Part II of Table V, the maximum water and mineral loss was found to occur in the day period. Urine volume, potassium, and chloride excretion reached a maximum during the day and the negative water balance of the day exceeded that of the night period. Sodium balance may be temporarily reversed, the excretion of the night period exceeding that of the day as it was found to be on the second day of the experiment.

The results of a fasting period of two days duration are presented in Table VI. The usual rhythm in water balance, chloride, and sodium excretion was maintained and the small variations in phosphate and sulphate noted under normal conditions were more pronounced. Nocturnal excretion of potassium, ammonia, and titratable acidity was increased on the second day of fasting to the point at which it exceeded that of the day. Sodium, potassium, and chloride were stored during the recovery period. In spite of the demand of the body for the replenishment of depleted stores, the diurnal rhythm in excretion of these substances persisted.

TABLE IV
The effect of changes in the routine of sleep and activity on the diurnal rhythm N F age 10 years Weight 27 kgm

Time	Activity	Water balance			Urine							Body tem perature	
		Total water avail- able	Total water loss	Balance	Volume	Specific gravity	Nitrogen	Cl	P	S	Na		K
6 A M - 6 P M	Active Sleeping	grams 833	grams 1093	grams -260	cc 843	1 009	grams 4 19	mgm 1795	mgm 435	mgm 390	mgm 1024	mgm 712	C
6 P M - 6 A M		832	736	+ 96	503	1 013	3 38	414	380	303	207	350	
6 A M -12 N	Flat on back Awake quiet Active Sleeping Active	416	519	-103	426	1 013	2 19	1045	178	221	702	480	36 8
12 N - 6 P M		416	506	- 90	329	1 012	1 98	570	168	240	316	390	37 2
6 P M -12 M N		416	360	+116	257	1 012	1 64	171	140	173	87	187	37 2
12 M N - 6 A M		416	456	- 15	341	1 013	2 16	446	161	212	266	357	37
6 A M -12 N	Sleeping Active Sleeping Sleeping	416	433	- 17	310	1 012	2 25	400	185	260	228	339	37 5
12 N - 6 P M		416	491	- 75	333	1 010	2 28	586	182	278	280	493	37 4
6 P M -12 M N		416	432	- 16	253	1 013	1 77	291	147	193	179	246	37 2
12 M N - 6 A M		416	380	+ 36	278	1 011	1 88	259	162	239	131	291	37 1

TABLE V
The influence of diuresis and dehydration on the diurnal rhythm H D, age 5 years Weight 20 kgm

Time	Activity	Body tem- perature	Water balance			Urine										
			Total water avail- able	Total water loss	Balance	Volume cc	Specific gravity	Nitrogen grams	N ₂ cc n/10	K cc n/10	Cl cc n/10	NH ₃ cc n/10	Titra- table acidity cc n/10			
			grams	grams	grams											
<i>Part I Diuresis</i>																
6 A M -6 P M	Active	37.5	1900	1900	+ 67	1520	1.006	3.68	139		274		173			
6 P M -6 A M	Sleeping	37.1	1890	1823		1140	1.004	3.07	97		110		148			
<i>Part II Dehydration</i>																
6 A M -6 P M	Active	37.1	751	907	- 156	621	1.010	3.43	206	180	315	158	152			
6 P M -6 A M	Sleeping	37.3	738	589	+ 149	253	1.021	2.96	120	78	137	157	145			
6 A M -6 P M	Active	37.4	208	561	- 353	212	1.030	3.55	198	196	295	131	157			
6 P M -6 A M	Sleeping	37.6	195	405	- 210	163	1.035	3.33	238	111	236	106	160			
6 A M -6 P M	Active	37.1	205	463	- 258	204	1.034	3.71	321	263	400	104	147			
6 P M -6 A M	Sleeping	37.6	200	284	- 84	165	1.038	3.81	247	173	247	105	162			

TABLE VI
The effect of fasting on the diurnal rhythm N F age 10 years Weight 27 kgm

Time	Period	Water balance				Spec- ific grav- ity	Nitro- gen grams	Inorganic acid				Beta oxy- bu- tyric cc % m/10	Titra- table acid cc % m/10	Inorganic base					NH ₄ cc % m/10	Inorganic base + titratable acidity + NH ₄ cc % m/10																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
		Total water avail- able grams	Total water lost grams	Bal- ance grams	Vol- ume cc			Cl cc % m/10	PO ₄ cc % m/10	SO ₄ cc % m/10	Total			Na cc % m/10	K cc % m/10	Ca cc % m/10	Mg cc % m/10	Total																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
6 A.M.-6 P.M.		906	1195	-289	962	1.010	3.63	344	252	202	798	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc	cc</

* No allowance was made for water freed through the catabolism of body glycogen. Preformed water freed by the tabolism of body protein and fat was calculated by the method suggested by Newburgh, Johnston, and Falcon Lesse (4)

The results of a study of a three day fast contained in Table VII show the same fluctuation in water, urine volume and sodium as in Table VI. Potassium balance was reversed on the second day of fasting, the excretion of the night period exceeding that of the day. The ability of the body to conserve base to replace depleted stores was strikingly

TABLE VII

The influence of a three-day fast and subsequent recovery period, on the diurnal rhythm
J R, age 15 years Weight 40 kgm

Time	Period	Water balance			Urine volume	Sodium		Potassium	
		Total water available*	Total water lost	Balance		Intake	Urine	Intake	Urine
		grams	grams	grams	cc	mgm	mgm	mgm	mgm
6 A M -6 P M	Fasting	1140	1745	-605	1225		2795		942
6 P M -6 A M		1140	1030	+110	675		1305		503
6 A M -6 A M		2280	2775	-495	1900		4100		1445
6 A M -6 P M	Fasting	1050	1510	-460	942		1141		332
6 P M -6 A M		1050	1200	-150	800		790		823
6 A M -6 A M		2100	2710	-610	1742		1931		1155
6 A M -6 P M	Fasting	1040	1460	-420	807		890		1242
6 P M -6 A M		1020	1070	- 50	690		400		1066
6 A M -6 A M		2060	2530	-470	1497		1290		2308
6 A M -6 P M	Stand- ard diet	910	1020	-110	565	230	80	610	562
6 P M -6 A M		910	1110	-200	490	230		610	94
6 A M -6 A M		1820	2130	-310	1055	460	80	1220	656
6 A M -6 P M	Stand- ard diet	910	880	+ 30	365	230		610	143
6 P M -6 A M		910	790	+120	405	230		610	61
6 A M -6 A M		1820	1670	+150	770	460		1220	204

* Preformed water freed by the catabolism of body protein and fat was calculated by the method suggested by Newburgh, Johnston, and Falcon-Lesses (4)

demonstrated during the recovery period. Sodium almost disappeared from the urine. Potassium although diminished was excreted in larger quantities during the day than at night. Whatever the factors may be controlling the diurnal fluctuations, it is obvious that each element may be selectively influenced by other more fundamental physiological mechanisms, in this case, the demand of the organism for sodium to replenish depleted stores.

DISCUSSION

The foregoing experiments furnish proof of a well established diurnal rhythm in water balance, urine volume, urinary sodium, potassium, and

chloride excretion, and specific gravity The small fluctuations noted in urinary phosphate, sulphate, titratable acidity, and ammonia are of questionable significance, although there is almost uniformly a larger excretion of all these solutes during the day than at night No significant change in calcium or magnesium occurs The increased specific gravity of the night urine specimen is due largely to the nitrogenous fraction in spite of the fact that the total amount of urinary nitrogen excreted is slightly smaller during the night

As a rule the negative water balance, urine volume, and urinary sodium, potassium, and chloride output reach a maximum in the morning period from 6 00 A M to 12 00 noon, decline in the afternoon, and decrease sharply at night, water balance becoming positive, and volume of urine, and sodium, potassium and chloride excretion reaching a minimum in the period from 12 00 midnight to 6 00 A M, which is coincident with the period of greatest water retention The results obtained are at variance with those of Norn (3) who found that the period of maximum excretion occurred from 12 00 noon to 3 00 P M They are in accord with those of Simpson (2) who observed a large matutinal increase in urinary volume and chloride after waking

Under standard experimental conditions the rhythm is tenaciously maintained, although in abnormal conditions, such as dehydration and fasting, various constituents may be specifically influenced, for example sodium in dehydration and potassium in fasting

Throughout the entire study a rough parallelism has been observed between water balance and the sodium, potassium, and chloride which appear in the urine, the negative water balance of the day period coinciding with the period of maximum urinary sodium, potassium, and chloride excretion, and the positive water balance of the night period coinciding with the period of minimum excretion The largest negative water balance and sodium, potassium, and chloride excretion occur together from 6 00 A M to 12 00 noon and the largest positive water balance and smallest mineral excretion from 12 00 midnight to 6 00 A M This is of interest in view of the fundamental conception of Gamble, Ross, and Tisdall (13) who established the fact that a close quantitative relationship exists between mineral and water metabolism, a retention of water requiring a retention of minerals and vice versa In the data recorded here only a qualitative rather than a quantitative relationship can be established This is not surprising, however, in view of the technical difficulties encountered in accurately determining water balance over the short periods used In general the results tend to uphold such a concept

Based on the fact that potassium is limited almost exclusively to intracellular fluid and sodium to extracellular fluid, Gamble, Ross, and Tisdall (13) have considered changes in potassium as indicative of changes

The results of a study of a three day fast contained in Table VII show the same fluctuation in water, urine volume and sodium as in Table VI. Potassium balance was reversed on the second day of fasting, the excretion of the night period exceeding that of the day. The ability of the body to conserve base to replace depleted stores was strikingly

TABLE VII

*Temperature of a three day fast and subsequent recovery period, on the diurnal rhythm
J. R., age 15 years Weight 40 kgm*

Time	Period	Water balance			Urine volume	Sodium		Potassium	
		Total water available*	Total water lost	Balance		Intake	Urine	Intake	Urine
		grams	grams	grams	cc	mgm	mgm	mgm	mgm
6 A M-6 P M	Fasting	1140	1745	-605	1225		2795		942
6 P M-6 A M		1140	1030	+110	675		1305		503
6 A M-6 A M		2280	2775	-495	1900		4100		1445
6 A M-6 P M	Fasting	1050	1510	-460	942		1141		332
6 P M-6 A M		1050	1200	-150	800		790		823
6 A M-6 A M		2100	2710	-610	1742		1931		1155
6 A M-6 P M	Fasting	1040	1460	-420	807		890		1242
6 P M-6 A M		1020	1070	- 50	690		400		1066
6 A M-6 A M		2060	2530	-470	1497		1290		2308
6 A M-6 P M	Stand ard diet	910	1020	-110	565	230	80	610	562
6 P M-6 A M		910	1110	-200	490	230		610	94
6 A M-6 A M		1820	2130	-310	1055	460	80	1220	656
6 A M-6 P M	Stand ard diet	910	880	+ 30	365	230		610	143
6 P M-6 A M		910	790	+120	405	230		610	61
6 A M-6 A M		1820	1670	+150	770	460		1220	204

* Performed water freed by the catabolism of body protein and fat was calculated by the method suggested by Newburgh, Johnston, and Falconer (4)

demonstrated during the recovery period. Sodium almost disappeared from the urine. Potassium although diminished was excreted in larger quantities during the day than at night. Whatever the factors may be controlling the diurnal fluctuations, it is obvious that each element may be selectively influenced by other more fundamental physiological mechanisms. In this case, the demand of the organism for sodium to replenish depleted stores.

DISCUSSION

The foregoing experiments furnish proof of a well established diurnal rhythm in water balance, urine volume, urinary sodium, potassium, and

chloride excretion, and specific gravity. The small fluctuations noted in urinary phosphate, sulphate, titratable acidity, and ammonia are of questionable significance, although there is almost uniformly a larger excretion of all these solutes during the day than at night. No significant change in calcium or magnesium occurs. The increased specific gravity of the night urine specimen is due largely to the nitrogenous fraction in spite of the fact that the total amount of urinary nitrogen excreted is slightly smaller during the night.

As a rule the negative water balance, urine volume, and urinary sodium, potassium, and chloride output reach a maximum in the morning period from 6:00 A.M. to 12:00 noon, decline in the afternoon, and decrease sharply at night, water balance becoming positive, and volume of urine, and sodium, potassium, and chloride excretion reaching a minimum in the period from 12:00 midnight to 6:00 A.M., which is coincident with the period of greatest water retention. The results obtained are at variance with those of Norn (3) who found that the period of maximum excretion occurred from 12:00 noon to 3:00 P.M. They are in accord with those of Simpson (2) who observed a large matutinal increase in urinary volume and chloride after waking.

Under standard experimental conditions the rhythm is tenaciously maintained, although in abnormal conditions such as dehydration and fasting, various constituents may be specifically influenced, for example sodium in dehydration and potassium in fasting.

Throughout the entire study a rough parallelism has been observed between water balance and the sodium, potassium, and chloride which appear in the urine, the negative water balance of the day period coinciding with the period of maximum urinary sodium, potassium, and chloride excretion, and the positive water balance of the night period coinciding with the period of minimum excretion. The largest negative water balance and sodium, potassium, and chloride excretion occur together from 6:00 A.M. to 12:00 noon and the largest positive water balance and smallest mineral excretion from 12:00 midnight to 6:00 A.M. This is of interest in view of the fundamental conception of Gamble, Ross, and Tisdall (13) who established the fact that a close quantitative relationship exists between mineral and water metabolism, a retention of water requiring a retention of minerals and vice versa. In the data recorded here only a qualitative rather than a quantitative relationship can be seen. It is not surprising, however, in view of the fact that the method used is not accurately determining water balance.

chloride excretion, and specific gravity. The small fluctuations noted in urinary phosphate, sulphate, titratable acidity, and ammonia are of questionable significance, although there is almost uniformly a larger excretion of all these solutes during the day than at night. No significant change in calcium or magnesium occurs. The increased specific gravity of the night urine specimen is due largely to the nitrogenous fraction in spite of the fact that the total amount of urinary nitrogen excreted is slightly smaller during the night.

As a rule the negative water balance, urine volume, and urinary sodium, potassium, and chloride output reach a maximum in the morning period from 6 00 A M to 12 00 noon, decline in the afternoon, and decrease sharply at night, water balance becoming positive, and volume of urine, and sodium, potassium, and chloride excretion reaching a minimum in the period from 12 00 midnight to 6 00 A M, which is coincident with the period of greatest water retention. The results obtained are at variance with those of Norn (3) who found that the period of maximum excretion occurred from 12 00 noon to 3 00 P M. They are in accord with those of Simpson (2) who observed a large matutinal increase in urinary volume and chloride after waking.

Under standard experimental conditions the rhythm is tenaciously maintained, although in abnormal conditions such as dehydration and fasting, various constituents may be specifically influenced, for example sodium in dehydration and potassium in fasting.

Throughout the entire study a rough parallelism has been observed between water balance and the sodium, potassium and chloride which appear in the urine, the negative water balance of the day period coinciding with the period of maximum urinary sodium, potassium, and chloride excretion, and the positive water balance of the night period coinciding with the period of minimum excretion. The largest negative water balance and sodium, potassium, and chloride excretion occur together from 6 00 A M to 12 00 noon and the largest positive water balance and smallest mineral excretion from 12 00 midnight to 6 00 A M. This is of interest in view of the fundamental conception of Gamble, Ross, and Tisdall (13) who established the fact that a close quantitative relationship exists between mineral and water metabolism, a retention of water requiring a retention of minerals and vice versa. In the data recorded here one is tempted to suggest a qualitative rather than a quantitative relationship can be seen. It is not surprising however, in view of the technical difficulties in accurately determining water balance that the results tend to uphold such


limited almost exclusively to plasma fluid, Gamble, Ross, and

chloride excretion, and specific gravity. The small fluctuations noted in urinary phosphate, sulphate, titratable acidity, and ammonia are of questionable significance, although there is almost uniformly a larger excretion of all these solutes during the day than at night. No significant change in calcium or magnesium occurs. The increased specific gravity of the night urine specimen is due largely to the nitrogenous fraction in spite of the fact that the total amount of urinary nitrogen excreted is slightly smaller during the night.

As a rule the negative water balance, urine volume, and urinary sodium, potassium, and chloride output reach a maximum in the morning period from 6 00 A M to 12 00 noon, decline in the afternoon, and decrease sharply at night, water balance becoming positive, and volume of urine, and sodium, potassium, and chloride excretion reaching a minimum in the period from 12 00 midnight to 6 00 A M, which is coincident with the period of greatest water retention. The results obtained are at variance with those of Norn (3) who found that the period of maximum excretion occurred from 12 00 noon to 3 00 P M. They are in accord with those of Simpson (2) who observed a large matutinal increase in urinary volume and chloride after waking.

Under standard experimental conditions the rhythm is tenaciously maintained although in abnormal conditions such as dehydration and fasting, various constituents may be specifically influenced, for example sodium in dehydration and potassium in fasting.

Throughout the entire study a rough parallelism has been observed between water balance and the sodium, potassium and chloride which appear in the urine, the negative water balance of the day period coinciding with the period of maximum urinary sodium, potassium, and chloride excretion, and the positive water balance of the night period coinciding with the period of minimum excretion. The largest negative water balance and sodium, potassium and chloride excretion occur together from 6 00 A M to 12 00 noon and the largest positive water balance and smallest mineral excretion from 12 00 midnight to 6 00 A M. This is of interest in view of the fundamental conception of Gamble, Ross, and Tisdall (13) who established the fact that a close quantitative relationship exists between mineral and water metabolism, a retention of water requiring a retention of minerals and vice versa. In the data recorded here not a quantitative rather than a quantitative relationship can be seen. It is not surprising, however, in view of the difficulty of accurately determining water balance that the results tend to uphold such



limited almost exclusively to the fluid Gamble, Ross, and Tisdall as indicative of changes

in intracellular water and changes in sodium as corresponding to those affecting extracellular water. Since sodium and potassium both take part in the diurnal rhythm, it may be assumed that both intra- and extracellular water and minerals enter into the changes noted.

In general the fluctuations recorded seem to be dependent more on the difference in the state of consciousness, as between sleeping and waking, than upon variations in the degree of physical activity. Although it is dangerous to draw conclusions from a single experiment, the data presented in Table IV would tend to minimize the importance of the latter. On the second experimental day, in spite of the fact that the subject remained quiet and inactive flat on his back from 6 00 A M to 12 00 noon, the usual matutinal increases in urine volume, chloride, sodium, and to a lesser extent potassium, occurred, whereas with the resumption of normal activity in the subsequent period from 12 00 noon to 6 00 P M, excretion was materially diminished. The results are in accord with those of Norn (3) who found the rhythm was uninfluenced by wide differences in muscular activity.

The mechanism involved remains obscure. Since the subjects were fed equal meals throughout the twenty-four hour period, differences in intake are excluded unless it be discovered that sleep interferes with intestinal absorption. That such is not the case is indicated by the fact that the rhythm persists in fasting.

The variations in the metabolic rate between sleeping and the waking state could account for only minor fluctuations. In the experiments recorded, the metabolic rate, estimated from the metabolism tables of Atwater and Benedict, was usually approximately 15 to 20 per cent higher during the day from 6 00 A M to 6 00 P M than at night. Such an increase obviously provides for excretion of larger amounts of minerals and water of hydration and oxidation of catabolized substances during the day than at night. The difference in regard to water, however, amounts to only a few cubic centimeters and is insufficient to account for the large diurnal rhythm noted. Further evidence of independence of metabolic rate has been presented in Table IV in which the usual matutinal increases occurred in spite of the fact that the subject remained flat on his back and inactive, whereas in the subsequent period, when with the resumption of normal activity the metabolic rate increased, the values for urine volume and mineral excretion decreased.

SUMMARY AND CONCLUSIONS

- 1 There is a diurnal rhythm in mineral and water balance characterized by the facts that urine volume and urinary sodium, potassium, and chloride excretion are greater during the day than at night. The larger urine volume of the day period is associated with a negative water balance, counterbalanced at night by a decreased urine volume and

positive water balance Only slight fluctuations are noted in urinary phosphate, sulphate, titratable acidity, and ammonia, although there is almost uniformly a larger excretion of all these solutes during the day than at night No significant shift in calcium and magnesium occurs "Total inorganic acid" and "total base" excretion are greater during the day than at night, due largely to the sodium, potassium, and chloride fractions

2 The negative water balance, urine volume, and urinary sodium, potassium, and chloride output reach a maximum in the morning period from 6:00 A M to 12:00 noon, decline in the afternoon, and fall off sharply at night, water balance becoming positive, and sodium, potassium, and chloride excretion reaching a minimum from 12:00 midnight to 6:00 A M, coincident with the period of greatest water retention

3 The rise in the specific gravity of the night urine is due largely to an increase in the molar concentration of the nitrogenous fraction, in spite of the fact that the total amount of urinary nitrogen excreted is slightly diminished during the night

4 A rough parallelism exists between water balance and urinary sodium, potassium, and chloride excretion The negative water balance of the day period coincides with the period of maximum sodium, potassium, and chloride excretion and the positive water balance of the night period coincides with the period of minimum excretion The largest negative water balance and sodium, potassium, and chloride excretion occur together from 6:00 A M to 12:00 noon and the largest positive balance and smallest excretion from 12:00 midnight to 6:00 A M

5 Since sodium and potassium both take part in the diurnal rhythm, it may be assumed that both intra and extracellular water and mineral metabolism contribute to the changes observed

6 Either urinary sodium or potassium excretion may be specifically influenced in abnormal metabolic conditions In dehydration the diurnal rhythm in sodium is temporarily reversed with night excretion exceeding that of the day period, while in fasting potassium may be reversed

7 The mechanism involved in maintaining such a constant rhythm seems to be dependent upon the difference between the sleeping and waking states of consciousness rather than upon changes in the degree of physical activity, although the influence of the latter has not been adequately excluded

BIBLIOGRAPHY

- 1 McQuarrie, I, Manchester R C, and Husted, C, *Am J Dis Child* 1932, xlii, 1519 Study of the Water and Mineral Balances in Epileptic Children I Effects of Diuresis, Catharsis Phenobarbital Therapy and Water Storage
- 2 Simpson, G E *J Biol Chem*, 1924, lxx 107 Diurnal Variations in the Rate of Urine Excretion for Two Hour Intervals Some Associated Factors

- Simpson, G E, J Biol Chem, 1929, lxxiv, 393 Changes in the Composition of Urine brought about by Sleep and other Factors
- Simpson, G E, J Biol Chem, 1926, lvi, 535 The Effect of Sleep on Urinary Chlorides and pH
- 3 Norn, M, Skandinav Arch f Physiol, 1929, lv, 184 Über Schwankungen der Kalium-, Natrium-, und Chlorid-ausscheidung durch die Niere im Laufe des Tages
 - 4 Newburgh, L H, Johnston, M W, and Falcon-Lesses, M, J Clin Invest, 1930, viii, 161 Measurement of Total Water Exchange
 - 5 Hawk, P B, and Bergheim, O, Practical Physiological Chemistry Blakiston's Son & Co, Philadelphia, 1926, 9th ed
 - 6 Fiske, C H, and Subbarow, Y, J Biol Chem, 1925, lxxi, 375 The Colorimetric Determination of Phosphorus
 - 7 Fiske, C H, J Biol Chem, 1921, lvi, 59 The Determination of Inorganic Sulfate, Total Sulfate, and Total Sulfur in Urine by the Benzidine Method
 - 8 Folin, O, Am J Physiol, 1905, xiii, 102 Laws Governing the Chemical Composition of Urine
 - 9 Folin, O, and Bell, R D, J Biol Chem, 1917, xlix, 329 Applications of a New Reagent for the Separation of Ammonia I The Colorimetric Determination of Ammonia in Urine
 - 10 Barber, H H, and Kolthoff, I M, J Am Chem Soc, 1928, l, 1625 A Specific Reagent for the Rapid Gravimetric Determination of Sodium
 - 11 Van Slyke, D D, and Sendroy, Julius, J Biol Chem, 1929, lxxiv, 217 Gasometric Determination of Oxalic Acid and Calcium and Its Application to Serum Analysis
 - 12 Shohl, A T, and Bennett, H B, J Biol Chem, 1928, lxxviii, 643 A Micro Method for the Determination of Potassium as Iodoplatinate
 - 13 Gamble, J L, Ross, G S, and Tisdall, F F, J Biol Chem, 1923, lvii, 633 The Metabolism of Fixed Base during Fasting

THE EFFECT OF SPLENIC CONTRACTION ON THE FORMED ELEMENTS OF THE BLOOD IN A CASE OF ANEMIA AND SPLENOMEGALY

BY D K MILLER AND C P RHOADS

(From the Hospital of the Rockefeller Institute for Medical Research New York City)

(Received for publication June 17 1933)

The function of the spleen as a reservoir for the formed elements of the blood is a comparatively recent conception. Experimental studies have dealt principally with the number of erythrocytes stored or static in the splenic pulp and have neglected other equally significant cellular elements. Such studies moreover have been almost entirely made upon animals and the application of the results to problems of human physiology has not been proven.

Stukeley (1) suggested in 1723 that the spleen manifested relaxation and active muscular contraction. Gray (2) in 1854 mentioned the function of the spleen as that of a 'safety valve' for the storage of blood apart from the general circulation. Roy was the first physiologist to observe the actual rhythmic movements and contraction of the spleen in laboratory animals (3). His work was confirmed in 1896 by Schafer and Moore who also demonstrated the innervation of the organ (4). The present day views of the function of the spleen as a blood reservoir are largely due to the work of Barcroft and his associates (5). Barcroft brought the spleens of dogs out through a slit in the abdominal wall and left them in this position for considerable periods of time protected only by saline dressings. With these preparations he demonstrated the rhythmic movements of the spleen and also the active contraction after various stimuli such as heat, exercise and emotion. It was shown in later experiments that the spleen of the dog contracts to one half or one third of its normal size during exercise and to an even greater extent after severe hemorrhage or the death of the animal. His work with carbon monoxide has clearly demonstrated that the spleen serves as a reservoir of red blood corpuscles.

Many studies have been made on the hemoglobin content and the erythrocyte count of blood expelled from the spleen both during active contraction and while the organ was quiescent. Consistent results have not been obtained. In some cases identical values were obtained for blood from the splenic artery and vein. Cruickshank (6) working in Barcroft's laboratory by cannulizing the inferior mesenteric vein col-

lected blood during contractions of the spleen. At the same time he measured the contraction of the spleen and the quantity of blood expelled. From experiments of this nature he determined that the blood expelled was often richer in hemoglobin than was the blood of the general circulation. The concentration of hemoglobin varied during the contraction, the maximum value being 20 to 40 per cent higher than that of the normal peripheral blood. The amount of blood expelled from the spleen by a single contraction was estimated to be 2.6 to 5.6 per cent of the total blood volume of the animal.

An opportunity for the detailed study of the spleen as a reservoir of blood was presented to us in a patient in which there was an anemia and splenomegaly. The spleen in this patient was easily demonstrable and could be visualized by the x-ray. It was observed that the spleen invariably contracted following the intravenous injection of liver extract, a therapeutic measure instituted with a view to relieving the anemia. The present paper deals with the changes in the number and character of the formed blood elements which took place during the splenic contractions induced by this and other methods.

METHODS

The general plan was to study the character and cellular composition of the peripheral blood before and after the induced contraction of the spleen, and to compare these findings with the changes in the size of the organ, as determined by x-ray.

Blood samples were taken by venipuncture both before and after the splenic contraction. The red and white corpuscles were enumerated in the usual way in these samples. Hemoglobin determinations were made by the Sahli acid hematin method, using calibrated tubes and standards. The mean corpuscular volume of the erythrocytes was determined with the Wintrobe hematocrit tube. Enumeration of the blood platelets was then done on blood obtained from a freely bleeding puncture of the ear lobe. Three per cent sodium citrate solution prepared freshly every day was used as a diluting fluid in the red corpuscle pipette. Great care was exercised to keep the sodium citrate solution and all the glassware free from dust and dirt. Fragility tests of the red corpuscles were done by the usual method, using sodium chloride solutions in dilutions varying from 0.52 to 0.28 per cent. Controls were made on normal bloods at each determination. Blood volume determinations by the vital red method of Rowntree (7) were made before and after splenic contractions. X-rays of the spleen were taken before the injection and at regular intervals following the contractions. The surface area of the splenic shadow was measured on these x-rays with a Keuffel and Esser planimeter. The upper pole of the spleen could not be definitely outlined; therefore identical points were taken on the ribs from which measurements were made.

OBSERVATIONS

Clinical summary. The patient, a 55 year old Puerto Rican woman, entered the hospital complaining of attacks of diarrhea and abdominal discomfort of four and a half years duration. A diagnosis of sprue had been

made fifteen years ago while the patient was a resident of Puerto Rico. Following her arrival in this country thirteen years ago the diarrhea ceased and the patient gained weight. There was no history of yellow fever, typhoid or malaria.

The present illness began about four and a half years ago when the patient noticed five or six loose bowel movements a day. These attacks lasted about three weeks and occurred once or twice a year. An abdominal mass was noticed on the left side of her abdomen three years ago. Six months before entry the patient noticed shortness of breath and pallor. Three months later a local doctor found a severe anemia and transfused the patient. Following this the patient improved and was well until one month before entry when her diarrhea and abdominal discomfort returned. At the same time her pallor was again noticed and she became short of breath on exertion.

Physical examination. The patient was markedly emaciated and had moderate pallor of her skin and mucous membranes. No lymphadenopathy was present. The heart and lungs were normal. The abdomen was moderately distended. A large hard mass was palpated on the left side of the abdomen. The lower edge of the mass extended to the level of the umbilicus; the medial edge extended to the mid abdominal line. A notch was felt in this medial border. The red blood count was 3 240 000, hemoglobin 71 per cent, white cell count 1250. The differential counts done with Wright's stained smears and by the supravital technique showed no abnormal cells. The fragility of the red blood cells was within normal limits. Gastric analysis showed no free hydrochloric acid after histamine expression.

For the treatment of the anemia liver extract was given intravenously. During the first injection which was of twenty minutes duration the patient complained of severe abdominal pain localized over the splenic area. Nausea and vomiting promptly occurred. Toward the end of the injection marked flushing of the face, neck, hands and arms was present. Immediately following the injection palpation of the abdomen revealed that the spleen, the lower edge of which had been just below the level of the umbilicus, had contracted in size until its lower edge was palpable about 10 cm. below the costal margin. Moreover, whereas the spleen had been hard before injection, it was found to be very soft in consistency after the shrinkage in size. This observation suggested the studies reported in this communication. Table I represents a typical chronological protocol of these studies.

The results obtained in a typical study using liver extract by intravenous injection as a stimulant to splenic contraction are shown in Table II. From this table it is evident that without essential change in the total blood volume there was a significant increase in the formed elements of the blood following a shrinkage in the size of the spleen. Twenty-four hours later the spleen had practically regained its original size. Figure 1 presents tracings of the splenic shadow taken from the x-rays and demonstrates the change of size of the spleen. The area of the spleen before injection was 90.3 sq. cm. Immediately after injection the spleen had contracted to an area of 32.3 sq. cm. Accompanying this contraction there was an increase of 520 000 red blood cells in the erythrocyte count. The hemoglobin increased 15 per cent. At the same time even more marked rises were found in the white count and

TABLE I
Chronological protocol

Time	Remarks
2 45	Palpation of abdomen reveals a firm hard spleen, the lower edge of which is at the level of the umbilicus
2 58	X-ray of spleen
3 00	First bleeding Blood pressure 120/70
3 03	Platelet count
3 05	11 0 cc 1 5 per cent vital red solution given intravenously
3 09	Bleeding for blood volume
3 10	Injection of 10 0 cc liver extract Eli Lilly (or 20 cc Parke Davis) begun Blood pressure 122/68
3 15	Patient complained of severe pain in abdomen, headache and nausea No vomiting
3 17	Marked flushing of face has occurred
3 20	Blood pressure 84/44
3 25	Injection completed Spleen palpable about 2 0 cm below the costal margin
3 26	X-ray of spleen
3 28	Bleeding
3 30	Platelet count
3 38	11 0 cc 1 5 per cent vital red solution given intravenously
3 42	Bleeding for blood volume
3 45	X ray of spleen
24 hours later	X ray of spleen

the platelet count, the increase of the former being 950 cells and of the latter 74,000. Determination of the blood volume after contraction of the spleen showed an increase of the total blood volume of only 200 cc. With this the plasma volume decreased from 65 to 60 per cent. There was also a decrease in the total plasma volume. The fragility of the red cells to sodium chloride solutions was not altered after contraction of the spleen.

TABLE II
Effects of contraction of the spleen induced by liver extract

	Area of spleen	Red count	Hemo- globin	White count	Mean corpus- cular volume	Color index	Platelet count	Blood pressure
	<i>sq. cm</i>	<i>millions</i>	<i>per cent</i>		μ^3			
<i>Patient</i>								
Before injection	90.3	3.72	70	1400	0.870	0.950	49,000	120/70
After injection	32.3	4.24	85	2350	0.865	1.00	123,000	95/60
24 hours later	89.1	3.67	69	1150	0.860	0.945	52,000	
<i>Control</i>								
Before injection		2.68	67	4850	1.26	1.25	152,000	115/65
After injection		2.57	66	4500	1.28	1.28	138,000	90/60
	Total blood volume		Total plasma volume			Hematocrit		
	<i>cc</i>		<i>cc</i>					
Before injection	3,800		2,520			65		
After injection	4,000		2,410			60		

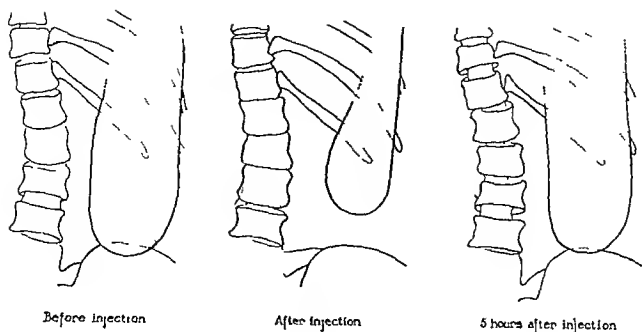


FIG 1

Shows a series of tracings taken from the X rays of the spleen demonstrating the size of the spleen immediately before the injection of liver extract immediately after contraction had occurred and 5 hours later at which time the spleen had practically regained its original size

A study of the formed elements of the blood following the injection of liver extract intravenously into a control patient with anemia, but without splenomegaly is also included in Table II. It is evident from these results that there was no appreciable alteration in these elements following this injection. Results similar to those found in Table II have been obtained repeatedly following the intravenous injection of liver extract into the patient with splenomegaly. The degree of contraction of the spleen and the changes in the formed elements of the blood have varied directly with the degree of contraction obtained in each study. The observations presented here are neither the maximal nor the minimal results obtained but are taken as the most typical.

The combined results of the alterations of the formed elements in the peripheral blood obtained after induced splenic contraction in five studies in which liver extract was used as the stimulant are presented in Table III. The average splenic contraction as shown was from a surface area of 93.6 sq. cm. to an area of 50.0 sq. cm. This is a decrease in the size of the spleen to 53 per cent of its original size. The average increase in the erythrocyte count after contraction was 500,000 red cells or an increase of 13.9 per cent. Together with this the hemoglobin increase in this series was 10 per cent, or an average increase of 13.7 per cent of the original average hemoglobin value. The white blood cells showed an average increase of 840 cells or average increase of 51 per cent. The blood platelets showed an average increase of 66,000 or of 105 per cent. It is apparent from this table that the relative increases were far greater for the white cells and the blood platelets than for the erythrocytes; this observation was seen in every study.



FIG. 2

This is an x-ray of the spleen taken immediately before the injection of liver extract. In this x-ray the lower border of the spleen is seen beneath the crest of the ilium.

TABLE III

Combined studies of splenic contractions induced by liver extract

	Area of spleen	Red count	Hemo- globin	White count	Platelet count
	<i>sq. cm.</i>	<i>millions</i>	<i>per cent</i>		
Before injection	93.6	3.62	73	1660	63,000
After contraction	50.0	4.12	83	2500	129,000
Per cent increase		13.9	13.7	51.0	105.0

These observations suggested the possibility of attempting to evaluate on the human spleen the effects of certain drugs which have been known



FIG. 3

This is an x ray of the spleen taken immediately after the injection of liver extract and demonstrates the change in size of the splenic shadow

to stimulate splenic contractions in animals. Consequently adrenalin, histamine and eserine were used to induce splenic contractions. The results obtained following the intramuscular injection of these drugs are presented in Table IV.

As shown in the table a marked contraction of the spleen was noted eight minutes after the intramuscular injection of 1.0 cc of adrenalin. The spleen contracted from an area of 109.0 sq. cm. to an area of 48.7 sq. cm. One hour later it had increased in size to 82.4 sq. cm. Accompanying this contraction there was an increase in the red blood cell count of 650,000 cells together with an increase of hemoglobin of 19 per cent. In this study the white blood cells showed the maximal increase obtained

TABLE IV

Effects of contraction of the spleen induced by adrenalin, histamine and eserine

	Area of spleen	Red count	Hemoglobin	White count	Mean corpuscular volume	Color index	Platelet count	Blood pressure
	sq. cm.	millions	per cent		μ^3			
<i>Adrenalin</i>								
Before injection	109.1	3.22	63	1500	0.917	0.985	90,000	124.75
8 minutes after injection of 1 cc. I.V.*	48.7	3.87	79	4100	0.925	1.01	158,000	138.80
1 hour after injection	84.2							
<i>Histamine</i>								
Before injection	100.8	3.32	65	1650	0.915	0.985	68,000	122.78
7 minutes after injection of 1 mgm. I.V.	75.5	3.67	70	2700	0.915	0.960	124,000	106.52
1 hour after injection	93.5							
<i>Eserine</i>								
Before injection	97.5	3.37	64	1250	0.900	0.955	96,000	128.70
40 minutes after injection of 2.4 mgm. I.V.	73.6	3.36	64	1100	0.890	0.955	88,000	115.75

* I.V. —intramuscularly

namely, 2,600 cells, or an increase of 172 per cent. With this contraction there was also an increase in the platelet count of 68,000.

Splenic contraction was also induced by the intramuscular injection of 1.0 mgm. of histamine. Seven minutes after this injection the spleen had contracted from 100.8 sq. cm. to 75.5 sq. cm. In one hour it had increased in size to 93.5 sq. cm., that is, almost to its original size. With this contraction there was an increase in the red cell count of 350,000 red cells, with a 5 per cent increase of hemoglobin. The white blood cells increased by 1,050 cells and the platelet count was increased by 56,000 platelets. As in the previous studies the white blood cells and platelets showed a greater proportional increase than the red cells and the hemoglobin.

The intramuscular injection of 2.4 mgm. eserine sulphate also induced a splenic contraction. This contraction did not occur until 40 minutes after the injection. The surface area of the spleen decreased from 97.5 sq. cm. to 73.6 sq. cm. As seen in Table IV there was no appreciable alteration in the formed elements of the blood accompanying this contraction. This was the only study in which no significant alteration of the formed elements of the blood was obtained with an induced splenic contraction. When the parasympathetic nervous system was blocked by giving atropine intramuscularly no contraction followed the injection of eserine.

Differential blood counts are found in Table V. There was a relative and absolute increase in the polymorphonuclear cell count after the

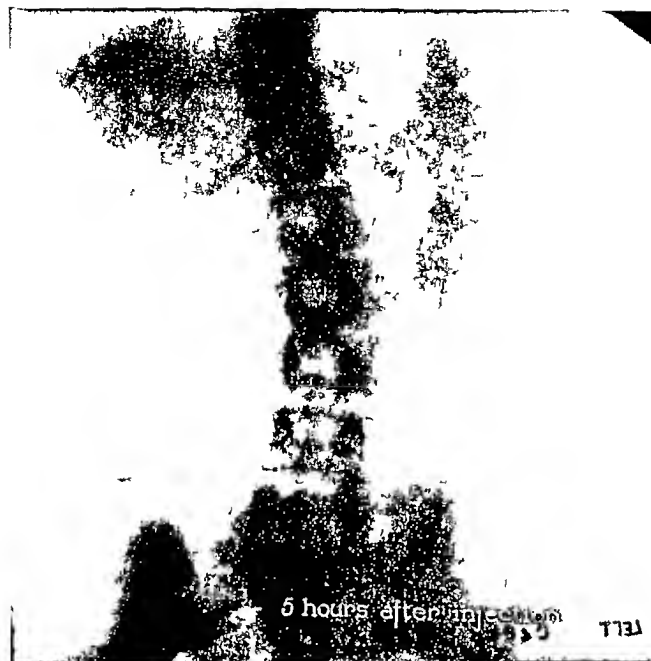


FIG. 4

This x-ray of the spleen was taken 5 hours after the contraction had occurred. It demonstrates the return of the spleen to its original size.

TABLE V

Differential white counts

	Contraction induced by liver extract		Contraction induced by adrenalin	
	Before	After	Before	After
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Polymorphonuclears	46	72	44	57
Eosinophils				1
Small lymphocytes	51	22	52	37
Large lymphocytes	2	2	2	2
Monocytes	1	4	2	3

induced splenic contraction. Accompanying this there was a relative decrease in the lymphocyte count. These variations were found with each splenic contraction studied. No abnormal cells were seen in the smears taken either before or after the contraction. The reticulocyte count showed no variation.

The blood pressure was followed in each study, as may be seen in Tables II and IV. A fall in blood pressure was invariably obtained following the intravenous injection of liver extract. A similar fall of blood pressure occurred after the injection of histamine. In the studies in which adrenalin and eserine were used as the stimulant to contraction, there was invariably a rise in blood pressure.

DISCUSSION

Before discussing the data presented in this paper it may be well to point out that while certain changes in blood cytology took place as a result of the induced splenic contractions, the results may not be indicative of the function of the normal spleen, since it may be recalled that the patient showed both an anemia and a splenomegaly. Obviously such an abnormal organ could store a much greater quantity of blood or cells than could a spleen of normal size. Furthermore, the degree or ratio of contraction obtained could scarcely be expected to occur with the normal spleen. Thus it might be thought that the pathological conditions might negate or partially invalidate the significance of these observations as regards the normal physiological function of the organ as a blood reservoir. This matter cannot be definitely settled at this time, but the authors prefer the interpretation that the present circumstances constitute merely an exaggeration of the normal.

The mechanism of the contractions is not clearly understood. Dale and his co-workers (8) isolated histamine from alcoholic extracts of fresh liver. Histamine present in the liver extract given intravenously might then be the substance responsible for the induction of the splenic contraction. Similar effects, namely a splenic constriction, were produced with liver extract and with a drug stimulating the sympathetic nervous system, adrenalin. In these two types of experiments, there was a lowering of the blood pressure with the liver extract and a rise in blood pressure accompanying the contraction induced by adrenalin. This would seem to eliminate the fall in blood pressure as a significant occurrence. Furthermore, whereas there occurred a peripheral vasodilation in the study with liver extract, and a peripheral vasoconstriction in the contraction induced with adrenalin, it may be safely stated that peripheral vasodilation had little or no effect on the production of the alteration in the blood elements.

The contraction induced by histamine may have been due to the direct action of the drug on the unstriated musculature of the organ or it may have been the response of the spleen to shock produced by the drug.

A drug which stimulates the parasympathetic nervous system namely, eserine, also gave a splenic contraction. It may be pointed out that the contraction was smaller than that obtained by other means, and that there was no alteration in the blood elements accompanying the change in size of the spleen.

Blood volume determinations showed very little alteration. There may have been at other times a greater increase in blood volume not detected by the method used. Vital red cannot be used repeatedly intravenously in clinical studies.

Perhaps the most striking fact among these results is the disproportionate increase in cell counts. Thus in each of the studies with liver extract and also with the other stimulating drugs the increase in the number of white blood cells was over threefold that of the red blood corpuscles, while the proportionate increase of the blood platelets was almost invariably greater than that of the white blood cells. No matter what mechanism was involved in the addition of these cells to the peripheral blood, these ratios may represent the relative proportions of these cells available in the splenic reservoir. This being the case the spleen may be considered as a potential and readily available source not only of red blood corpuscles but of white blood cells and blood platelets as well. It is not inconceivable that the spleen functions as a reservoir for these cellular elements.

SUMMARY

1 In a patient who showed both anemia and a splenomegaly it was possible to induce [and observe] a marked contraction of the spleen by the intravenous injection of liver extract and the intramuscular injection of adrenalin, histamine and eserine. This contraction was observed.

2 Accompanying these induced splenic contractions there occurred marked increases in the number of cellular elements of the circulating blood. The relative proportion of the various cellular components suggests that the blood of the splenic reservoir has a cytological composition in which the relative proportion of cells is different from that of the circulating blood.

3 These experiments make it obvious that the spleen must be considered not only as a reservoir of red blood cells, but that it can also store proportionally greater numbers of white blood cells and blood platelets.

BIBLIOGRAPHY

- 1 Stukeley, W. On the Spleen. Its Description and History. Uses and Diseases. London 1723. Quoted by McNee, J. W. *Lancet* 1931, i, 951. The Spleen. Its Structure, Functions and Diseases.
- 2 Gray, Henry. On the Structure and Use of the Spleen. Parker, London 1854, p. 380.
- 3 Roy, C. S. *J. Physiol.* 1881, iii, 203. The Physiology and Pathology of the Spleen.

- 4 Schifer E A and Moore, B I *Physiol* 1896 xx 11 On the Contractility and Innervation of the Spleen
- 5 Barcroft, J and Barcroft, H, J *Physiol*, 1923, lvi, 158 Observations on the Taking up of Carbon Monoxide by the Hemoglobin in the Spleen
Barcroft J, Murry, C D, Orshovats D Sands J and Weiss R, J *Physiol*, 1925 lx, 79 The Influence of the Spleen in Carbon Monoxide Poisoning
Barcroft, J, *Lancet*, 1925, i 319 Recent Knowledge of the Spleen
Barcroft, J and Stephens, J G, J *Physiol*, 1927, lvi, 1 Observations upon the Size of the Spleen
Barcroft, J and Nisimaru, Y, J *Physiol*, 1932 lxxiv, 299 Cause of Rhythmical Contraction of the Spleen
- 6 Cruickshank E W H, J *Physiol* 1926 lvi 455 On the Output of Hemoglobin and Blood by the Spleen
- 7 Rowntree, L G and Brown, G E, *The Volume of the Blood and Plasma in Health and Disease* W B Saunders Co Philadelphia 1929, p 219
- 8 Best C H, Dale, H H, Dudley, H W and Thorpe W A J *Physiol* 1927, lvi, 397 The Nature of the Vaso Dilator Constituents of Certain Tissue Extracts

THE PROTEINS OF BLOOD AND SUBCUTANEOUS LYMPH IN DOGS

By A A WEECH E GOETTSCH AND E B REEVES

(From the Department of Diseases of Children of Columbia University College of Physicians and Surgeons New York City)

(Received for publication June 17 1933)

In the course of experiments undertaken to discover the effect of the flow and the composition of lymph on the formation of edema an opportunity has been afforded for comparing the albumin and globulin concentrations of blood serum with those of lymph serum obtained from lymphatics of the extremities of dogs The comparison forms the basis of this communication

METHODS

The dogs used were of mongrel breeds and varied in weight from 15 to 26 kilograms Lymph was obtained through cannulas inserted into one of the lymphatic trunks just above the ankle of either a front or a hind leg Cannulization was performed sometimes under ether anesthesia, sometimes under nembutal anesthesia and sometimes with the aid of a local anesthetic (novocaine) only With animals under general anesthesia the flow of lymph was promoted by gentle massage of the foot and ankle and passive motion of the extremity The non anesthetized dogs were prepared by the method described by White Field and Drinker (1) and allowed to walk about the corridors the normal motion of the extremity furnishing the stimulus for lymph flow A fine fibrin clot which formed in the lymph on standing was removed before analysis Blood was obtained by puncture of the femoral artery usually at the end of the period of lymph collection and the serum separated as soon as possible In several instances blood serum obtained both before and after the collection of lymph failed to indicate any change in composition during the experiment

Methods of chemical analysis have been described in a previous paper (2) In some instances, because the quantities of lymph were limited nonprotein nitrogen was determined on the serum only and the value so obtained used in calculating the lymph proteins The error involved in such an assumption is small In nine instances in which parallel determinations were made of lymph and serum nonprotein nitrogen the average value for lymph was 24.5 mgm per 100 cc and for serum 22.7 mgm per 100 cc

ANALYTICAL RESULTS

The results of 25 parallel estimations of lymph and serum albumin and globulin made on specimens obtained from 13 dogs are shown in Table 1. A comparison of the albumin-globulin ratios is shown graphically in Chart 1. Table 1 shows that the A/G ratios for serum varied from 0.5 to 2.0. The results may be regarded as accurate at least to the first decimal as the relatively high protein content of serum means that small analytical errors are not reflected in significant variations in the A/G ratio. On the other hand the A/G ratios for lymph varied between 0.1 and 3.0. In this case several specimens of lymph were encountered

TABLE 1

Proteins of blood serum and lymph from the legs of dogs

Data grouped by experiments and arranged roughly in ascending order of the total protein content of lymph

Dog number	Albumin		Globulin		Total protein		Albumin/Globulin		Remarks*
	Serum	Lymph	Serum	Lymph	Serum	Lymph	Serum	Lymph	
	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc			
5	1.36	0.05	1.88	0.17	3.24	0.22	0.72	0.29	I oc—RI—Nutritional edema
839	2.36	0.03	3.18	0.29	5.54	0.32	0.74	0.10	Nem—RI
6	1.40	0.07	1.40	0.08	2.80	0.15	1.00	0.99	Nem—I γ —Phasmapheresis edema
	1.40	0.24	1.40	0.08	2.80	0.32	1.00	3.00	R α —Gentle massage
	1.40	0.28	1.40	0.41	2.80	0.69	1.00	0.69	R α —Vigorous massage
895	3.57	0.30	1.83	0.15	5.40	0.45	1.95	2.00	Nem—RI
	3.57	0.48	1.83	0.27	5.40	0.74	1.95	1.78	I γ
1000	2.34	0.21	3.36	0.57	5.70	0.78	0.70	0.37	E—3 hours after ether Dog walking
949	2.99	0.53	3.05	0.50	6.04	1.03	0.98	1.06	Nem—L α —Veins constricted
	2.99	0.44	3.05	0.29	6.04	0.73	0.98	1.52	L α —Veins not constricted
58	3.44	0.49	2.26	0.30	5.70	0.79	1.52	1.63	E—R α —Massage while recovering
	3.44	0.64	2.26	0.37	5.70	1.01	1.52	1.73	R α —Dog walking
	3.44	0.60	2.26	0.30	5.70	0.90	1.52	2.00	R α —Dog running
	3.44	0.60	2.26	0.34	5.70	0.94	1.52	1.77	R α —Walking after rest period

TABLE 1 (continued)

Dog number	Albumin		Globulin		Total protein		Albumin Globulin		Remarks*
	Serum	L ₃ mph	Serum	L ₃ mph	Serum	L ₃ mph	Serum	L ₃ mph	
	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc			
69	3.50	0.68	2.27	0.58	5.77	1.26	1.54	1.17	E—After recovery dog walking
840	3.08	0.97	1.99	0.52	5.07	1.49	1.55	1.86	Loc—Rl—Massage
	3.08	0.96	1.99	0.45	5.07	1.41	1.55	2.13	Rl—Walking
	3.08	1.16	1.99	0.49	5.07	1.65	1.55	2.37	Rl—Running
	3.08	0.86	1.99	0.41	5.07	1.27	1.55	2.10	Rl—Walking after rest period
91	3.75	1.15	2.63	0.74	6.38	1.89	1.43	1.55	Loc—La—Mixture walking and running
841	3.82	0.87	3.01	0.68	6.83	1.55	1.27	1.28	Nem—Ra
	3.82	1.14	3.01	0.91	6.83	2.05	1.27	1.25	La
	3.82	1.35	3.01	0.82	6.83	2.17	1.27	1.22	Rl and Ll
842	3.54	1.73	3.86	1.50	7.40	3.23	0.92	1.15	Nem—La—Veins constricted
950	2.49	0.85	5.40	2.60	7.89	3.45	0.46	0.33	Nem—La

* Abbreviations as follows *Nem* = nembital anesthesia *E* = ether anesthesia *Loc* = local novocaine anesthesia *La* = left fore leg *Ra* = right fore leg *Ll* = left hind leg *Rl* = right hind leg

in which the total protein concentration was less than 0.5 gram per cent and it is evident that small analytical errors in the analysis of such specimens produce wide variations in the A/G ratios. Moreover the ratios will increase in accuracy as the protein content of the specimen rises.¹ This factor of the accuracy of measurement has been given consideration in Chart 1. Collectively considered there were 10 instances in which the serum ratio was greater than the lymph ratio and 15 examples of the reverse relationship. The average albumin globulin ratio of all the lymph specimens analyzed was 1.41 ± 0.09 and the

¹ An example will render the magnitude of the possible analytical error more clear. Duplicate analyses of a lymph specimen show for total protein 0.38 and 0.42 gram per cent and for albumin 0.18 and 0.22 gram per cent. The A/G ratio calculated from the average of the duplicate measurements is 1.00 and yet the individual measurements can be combined to give ratios which vary from 0.75 to 1.38. An analytical variation of the same magnitude occurring in serum with a protein content of 6.00 grams per cent produces a ratio fluctuation between 0.98 and 1.02 only.

Probable error of the mean

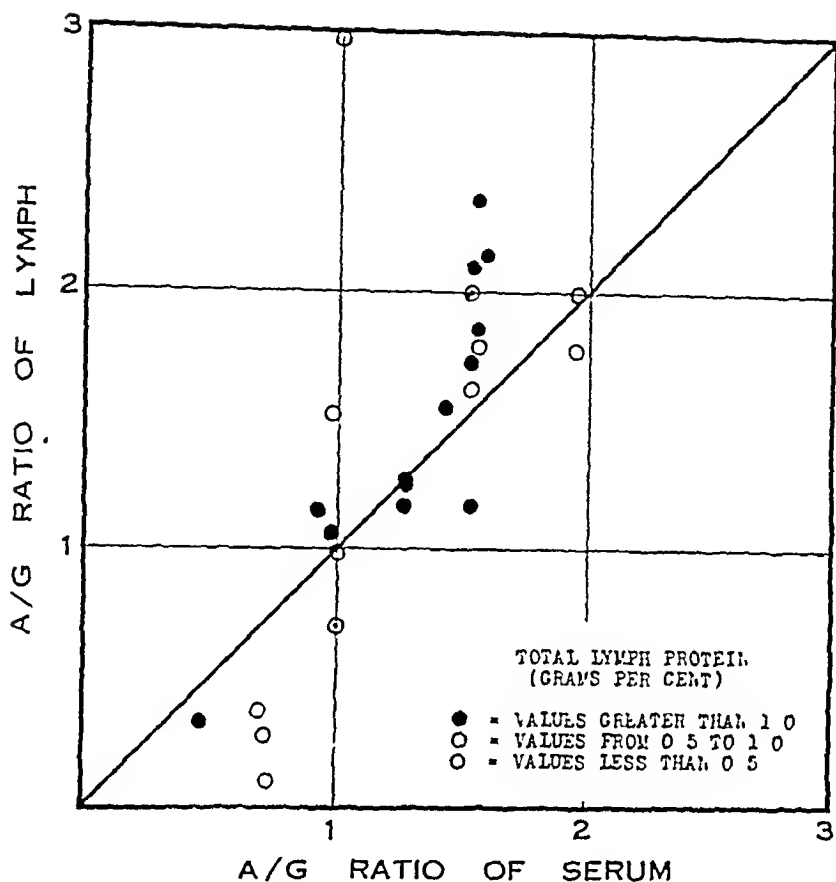


FIG. 1 RELATIONSHIP BETWEEN THE ALBUMIN GLOBULIN RATIOS OF BLOOD SERUM AND LYMPH FROM THE LATEMITHS IN DOGS

The diagonal line follows the course of identity in the ratios and serves to emphasize the divergence of individual determinations from this identity

corresponding average for the serums was 1.26 ± 0.05 . A statistical calculation by the product-moment formula gives for the respective ratios a coefficient of correlation of 0.81 with a probable error of 0.05. The correlation is sufficiently high to demonstrate a definite relationship between the albumin globulin ratios of lymph and serum.

DISCUSSION

An examination of pertinent literature, tabulated exhaustively by Drinker and Field (3), discloses a great number of observations of the total protein content of lymph while at the same time revealing a paucity of information concerning the protein fractions which enter into its composition. In 1891 Munk and Rosenstein (4) reported two analyses of lymph obtained from the leg of an 18-year-old girl who was afflicted with progressive elephantiasis and who presented a permanent lymph fistula over the upper portion of her calf. Both analyses revealed a total

protein concentration of 3.5 grams per cent, in one the albumin globulin ratio was 4 and in the other 2.4. Simultaneous analyses of the serum proteins were not made. In 1906 Morawitz (5), in attempting to obtain information concerning the origin of the plasma proteins, made two experiments on dogs in which the proteins of plasma were compared with those of thoracic duct lymph. In the first experiment the blood serum showed an albumin globulin ratio of 1.25 and the lymph serum a ratio of 1.56, in the second experiment the ratio for blood plasma was 1.44 and for lymph plasma 1.58. Although in both instances the lymph ratios were slightly higher than the blood ratios, the author did not regard the differences as significant. Finally in 1932 Wells (6) reported nine experiments in which the albumin globulin ratios of serum were compared with those of lymph obtained from the mesenteric lacteals of dogs. The measurements revealed an average albumin globulin ratio for serum of 1.18, an average ratio for lymph of 1.39, and agreed satisfactorily with direct estimations of the colloidal osmotic pressure. The analyses reported here are the first in which the protein fractions of serum have been compared with those of lymph from vessels which drain the subcutaneous tissues. Measurements of the colloidal osmotic pressure of serum and cervical lymph have, however, been reported by Loëwen, Field and Drinker (7). The osmotic pressures per gram of lymph protein varied from 55 to 103 mm. of water and in each instance were considerably higher than the corresponding pressures for serum protein. The excess pressure of lymph was thought to be an expression of greater permeability of the blood capillaries for serum albumin. An approximate calculation made by us on the basis of Govaerts' factors (8) indicates that several of the recorded pressures for cervical lymph are so high that they could not be accounted for even if all of the lymph protein were in the form of albumin. To explain them one would have to suppose either that lymph protein did not arise from serum protein or that the blood capillaries exhibit selective permeability for the smaller molecules of the albumin complex. A selective permeability of this nature is not in agreement with the analyses reported here.

The data reported in this paper show definitely that the albumin globulin ratios of serum and lymph are mutually dependent variables and are thus in accord with the view of Drinker and Field (3, 9) that lymph protein is derived from blood protein. The correlation between the two variables is sufficiently high to indicate that the serum ratio is at least a major factor in establishing the lymph ratio. It does not exclude the possibility that minor factors, among them a slight degree of selective permeability on the part of the blood capillaries, may prevent identity in the proportions of proteins in the two fluids. Several of the grouped analyses obtained in single experiments in this study (particularly with dogs 58 and 840) strongly suggest the modifying influence of

inquiry concerning the distribution of the capillary filtrate. We have already suggested that a large proportion of capillary tufts may be impermeable to protein. These tufts will yield a protein-free ultrafiltrate, the volume of which will depend upon the balance of pressures operating across their walls, namely, the colloidal osmotic pressure in serum together with capillary blood pressure on the inside of each tuft and mechanical tension in the intercellular spaces on the outside. The latter tension may be suspected of varying under conditions which affect the mobility of fluid in adjacent lymphatics, being decreased when the pumping action of the lymphatic valves is stimulated by exercise or massage and increased when the valves are at rest. The factors controlling the volume of a filtrate are, therefore, multiple and it is easy to conceive of circumstances, some pathologic and others purely physiologic, under which it will increase greatly or practically vanish. For present purposes it is important to hold in mind that this group of capillary tufts will be surrounded by an interstitial fluid which is essentially protein free and that some of this fluid, at times much and at times little, will find its way into radicles of the lymphatic system. Across the walls of other capillaries, those for example which permit the passage of protein, the course of events will be considerably modified. These vessels will yield interstitial fluid rich in protein which will act to diminish the effective osmotic pressure of the intracapillary colloids and therefore to increase the volume of filtrate. This filtrate will likewise find its way into adjacent radicles of the lymphatic system. To what extent diffusion through neighboring intercellular spaces will occur is a matter of pure conjecture but it may be supposed that so long as the lymphatics offer the path of least resistance the major portion of filtrate will move by this route. Among a large group of lymphatic capillaries we may therefore imagine that many are carrying a centripetal stream of lymph of extremely low protein content and that a few are transporting fluid the protein concentration of which approaches that of the serum. The rate of lymph flow from the various radicles will, moreover, be more rapid in those which contain fluid of high protein concentration. Lymph, however, collected from any of the larger lymphatic trunks will represent a mixture of the streams coming from different radicles. Its composition may be expected to vary considerably as regards total protein content but the proportions of the several protein fractions should remain roughly the same as those in the serum of the same animal. An exceptional blood capillary which exhibited selective permeability for albumin molecules could produce a minute flow of this protein fraction into the general stream and so raise the A/G ratio slightly above that in the blood. The theory then is consistent with the experimental observations being reported. It cannot, however, be reconciled with the readings of colloidal osmotic pressure of Loewen, Field, and Drinker (7) which indicate marked divergence between the A/G ratios of serum and lymph.

It will have been observed that the first theory, as outlined previously, can be identified in essential respects with the second if one supposes each individual blood capillary loop to possess pores the sizes of which cover the whole range of permeability which is assumed in the second theory for many capillaries. The distinction, however, is of fundamental importance for in the former instance one must imagine an interstitial fluid everywhere identical in composition with lymph and in the latter it can be thought that one very important function of the lymphatic system is to prevent interstitial fluid from attaining the relatively high protein concentrations of lymph. It is for this reason, chiefly, that the two theories have been outlined. Although either is consistent with the results reported, it seems desirable to insist that the available experimental evidence does not yet justify the conclusion that lymph as it is collected from a large lymphatic trunk is identical in chemical composition with interstitial fluid in the major portion of the region drained by the trunk.

SUMMARY

1 Experiments are reported in which the albumin and globulin concentrations in the serum of the blood are compared with those in lymph obtained from lymphatics of the legs of dogs.

2 In 25 parallel estimations of lymph and serum albumin and globulin the respective A/G ratios showed a correlation coefficient of 0.81 with a probable error of 0.05. The correlation is sufficiently high to demonstrate a definite relationship between the ratios of lymph and serum.

3 Two theories to account for the entrance of serum proteins into the lymphatics are discussed. The first supposes general permeability of the entire capillary network of the blood for protein and leads to the conclusion that interstitial fluid is everywhere identical in composition with the lymph which drains the area. The second assumes that occasional capillaries only permit the passage of protein, that this protein enters adjacent lymphatics at once, and that it never becomes disseminated throughout the intercellular spaces. The available evidence, including the findings in this investigation, although consistent with either theory, does not justify the conclusion that interstitial fluid and lymph are identical.

The authors wish to express their thanks to Dr Cecil K. Drinker and Dr Madeleine E. Field for their kindness in demonstrating the technique of cannulating lymphatics.

BIBLIOGRAPHY

- 1 White, J. C., Field, M. E. and Drinker, C. K., *Am. J. Physiol.*, 1933, **111**, 34.
On the Protein Content and Normal Flow of Lymph from the Foot of the Dog.

- 2 Weech, A A , Snelling, C E , and Goettsch, E , J Clin Invest , 1933, vii, 193 The Relation Between Plasma Protein Content, Plasma Specific Gravity and Edema in Dogs Maintained on a Protein Inadequate Diet and in Dogs Rendered Edematous by Plasmapheresis
- 3 Drinker, C K , and Field, M E , Lymphatics, Lymph and Tissue Fluid Williams and Wilkins Co , Baltimore, 1933
- 4 Munk, I , and Rosenstein, A , Virchow's Arch f path Anat , 1891, cxviii, 230 Zur Lehre von der Resorption im Darm, nach Untersuchungen an einer Lymph(chylus-)fistel beim Menschen
- 5 Morawitz, P , Beitr z chem Physiol u Path , 1906, vii, 153 Beobachtungen über den Wiederersatz der Bluteiweisskörper
- 6 Wells, H S , Am J Physiol , 1932, ci, 421 The Concentration and Osmotic Pressure of the Proteins in Blood Serum and in Lymph from the Lacteals of Dogs
- 7 Loewen, D F , Field, M E , and Drinker, C K , Am J Physiol , 1931, xcvi, 70 The Colloid Osmotic Pressure of Dog Blood and Lymph
- 8 Govaerts, P , Compt rend Soc de biol , 1925, xciii, 441 Influence du rapport albumines-globulines sur la pression osmotique des protéines du sérum
- 9 Drinker, C K , and Field, M E , Am J Physiol , 1931, xcvi, 32 The Protein Content of Mammalian Lymph and the Relation of Lymph to Tissue Fluid

PERMEABILITY OF CAPILLARIES TO PLASMA LIPOIDS

BY EVELYN B MAN AND JOHN P PETERS

(From the Department of Internal Medicine Yale University School of Medicine
New Haven)

(Received for publication June 19, 1933)

It has been rather generally assumed, chiefly because normal urine is practically free from lipoids (9, 10, 11) that the capillaries are impermeable to plasma lipoids. However, no serious attempts seem to have been made to test this assumption, in spite of its important bearing on the question of plasma colloid osmotic pressure. In order to determine whether lipoids diffuse through the capillaries without simultaneous migration of proteins, two types of experiments have been conducted. 1. Serum lipoids of normal individuals have been measured before and after the volume of the blood has been decreased, by a procedure which has been employed by Thompson, Thompson and Dailey (25), Krogh, Landis and Turner (13) and Ni and Rehberg (19). They demonstrated that if a normal person stands absolutely still for about half an hour the blood volume decreases and the serum proteins increase by about 10 per cent because there is transudation into the tissues of the lower extremities of a protein free ultrafiltrate of blood plasma. 2. Besides, body fluids have been analyzed for lipoids and proteins.

METHODS AND MATERIALS

The blood serum of five normal persons was analyzed for proteins, total fatty acids, lipid phosphorus and cholesterol after they had reclined for half an hour and again after they had stood still for half an hour. All the experiments except one were performed before breakfast. Blood was drawn from the antecubital vein with a minimum of stasis under oil. In all cases except that of P. L. the second blood sample was taken while the subject was standing. In the second group of experiments, the protein and lipid contents of ascitic, pericardial and pleural fluids were measured. Blood and fluids were collected simultaneously, in most instances before breakfast. All fluids were centrifuged to remove cells and fibrin clot from the aqueous suspension. Proteins were determined by Howe's modification of the Kjeldahl method (5), total fatty acids by the Man and Gildea (16) modification of the Stoddard and Drury technique, cholesterol by a gravimetric digitonin method (17), lipid phosphorus by a modification of the Fiske and Subbarow method (17).

RESULTS OF STANDING EXPERIMENTS

In Table I under each constituent the paired figures in the first

TABLE I
Standing experiments

Subject	Date		Lipoid P		Fatty acids from titer		Cholesterol		Proteins	
			mgm per cent	change per cent	m Eq	change per cent	mgm per cent	change per cent	grams per cent	change per cent
P L	January 11, 1933	Before	8.4		12.2		199		6.30	
		After	9.8	116.5	14.3	116.5	235	118.1	7.47	116.8
J H	January 23, 1933	Before	9.0		11.6		205		7.05	
		After	9.6	107.2	13.1	113.2	226	110.3	7.84	111.2
E M	January 3, 1933	Before	8.9		11.7		223		6.91	
		After	10.0	112.6	13.2	113.5	246	110.2	7.83	112.8
E M	February 17, 1933*	Before	9.9		12.6		209		7.00	
		After	10.8	108.6	14.0	111.3	231	110.5	7.91	113.0
C R	January 28, 1933	Before	10.4		16.2		229		6.33	
		After	10.7	102.1	16.0	98.8	260	113.5	6.99	110.5
C R	February 28, 1933	Before	9.7		12.2				6.31	
		After	10.2	105.5	13.5	110.7			7.10	112.6
T K	February 1, 1933	Before	8.4		9.9		168		6.52	
		After	9.6	114.1	11.7	118.6	183	109.0	7.27	111.4

* Second blood taken four and one quarter hours after a breakfast which contained approximately 19 grams of fat

columns represent the values observed before and after standing, the single figures in the second columns the degree to which the given substance in the serum was concentrated. The significance of "fatty acids from titer" is discussed more fully in the article describing the Van and Gildea modification of the Stoddard and Drury method (16). They found that saponification with potassium hydroxide as in the blood serum method, yielded only 82 per cent of the theoretical fatty acids from reprecipitated Pfanstiehl "pure" lecithin. Consequently, in order to calculate "total fatty acids," there was added to the "fatty acids from titer" 18 per cent of the phospholipoid fatty acid estimated on the assumption that two equivalent weights of fatty acid unite with one combining weight of phosphorus in lecithin and cephalin. Non-phospholipoid fatty acids may be calculated by subtracting from the "total fatty acids" the phospholipoid fatty acids estimated from the serum lipid phosphorus content. Values for "fatty acids from titer" have been presented here rather than those for non-phospholipoid fatty acids.

because it is recognized that these calculations may introduce a considerable error if the analytical errors of the two methods are cumulative, and because neither the exact chemical composition of phosphatides, nor the proportions of different phosphatides present in serum are known.

Cholesterol, without any exceptions, increased on standing in the same proportion as did the serum proteins, an indication that it is not diffusible. Fatty acids and lipid phosphorus in 5 of the 7 experiments (P L, J H, E M, January 1 and February 17, and C R, February 28) increased in the same proportions as the proteins. On the other hand, in the serum of T K these constituents increased more than the proteins, and in the serum of C R on January 28 they did not change. These divergent responses may be explained by the assumption that the metabolism of fat of these subjects was not in a state of equilibrium. The change in T K's blood sugar, from 76 mgm per cent to 85 after standing, suggests that the metabolism of carbohydrate was not uniform throughout the experiment. In contrast to the normal serum fatty acids in the first blood samples of P L, J H, and E M, the fatty acids both of C R on January 28 and of T K were at the extremes of the normal range of serum lipids. C R's total fatty acids on January 28 were the highest of 20 normal subjects and were appreciably greater than they were on February 28. After standing, his fatty acids and phospholipoids did not increase. T K's serum fatty acids, on the other hand, which were among the lowest of the normal values, increased proportionately more than his proteins. From these data it may be inferred, though it is not proven, that C R on January 28 was utilizing fat derived from his blood stream, while T K was mobilizing fat into his blood stream. An attempt was made to test this hypothesis by carrying out a similar standing experiment on a subject after a meal containing fat. However, the results of the experiment on E M on February 17 did not differ from those secured in the postabsorptive state, owing, perhaps, to the fact that too little fat was given.

In these experiments, then, cholesterol without exception, phosphatides and compounds of fatty acids in five of seven instances, behaved like non diffusible serum proteins.

Comparisons of transudates and serum

Data concerning the lipid content of transudates are presented graphically in the three figures. In Figure 1 the protein contents of ascitic, pericardial and pleural fluids are plotted on the ordinate, cholesterol content on the abscissa. The column above each point, at the top of the figure represents the concentration of cholesterol in the blood serum. The scale for serum cholesterol is $1/25$ of that for transudate cholesterol. Irregular variations in the lengths of the columns show that the amounts of cholesterol in the fluid bear no direct relation to the

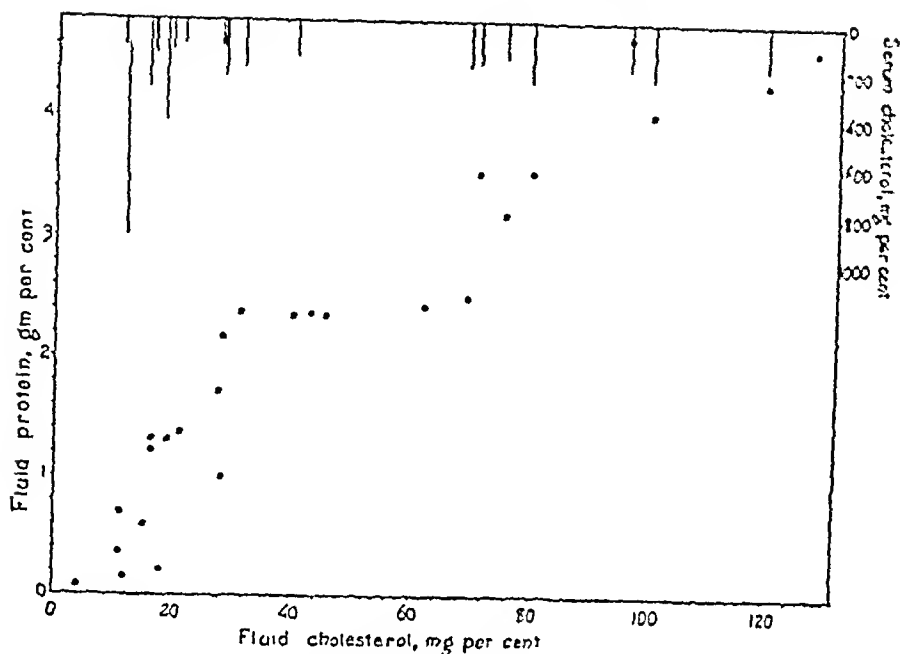


FIG 1 COMPARISON OF CHOLESTEROL AND PROTEIN IN TRANSUDATES

The vertical lines above indicate the concentrations of cholesterol in the sera of the patients from whom the transudates were secured

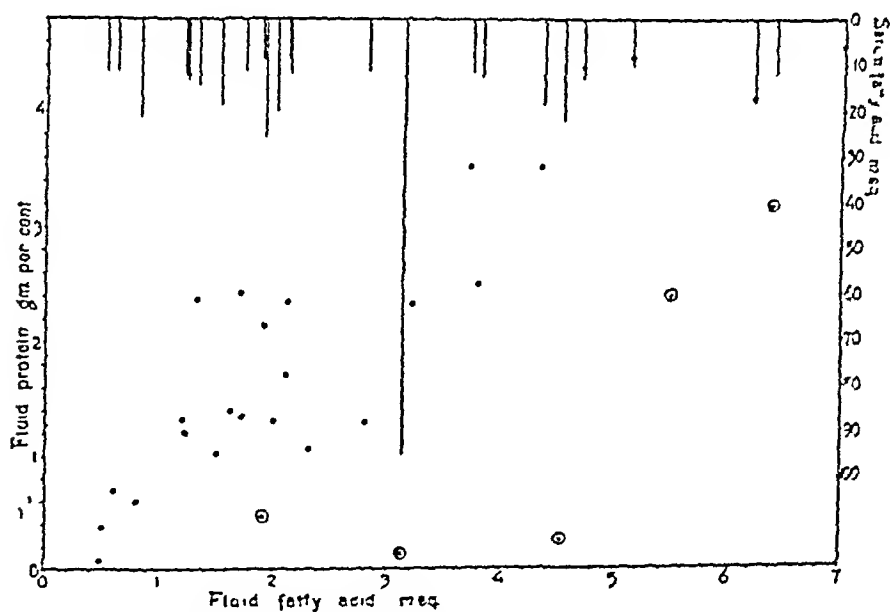


FIG 2 COMPARISON OF FATTY ACID AND PROTEIN IN TRANSUDATES

The vertical lines above indicate the concentrations of fatty acids in the sera of the patients from whom the transudates were secured. The points surrounded by circles are from patients whose sera contained little protein in proportion to fatty acids

concentrations of cholesterol in the serum. There is an obvious tendency for variations of protein and cholesterol contents of fluids to parallel one another.

The arrangement of Figure 2 is similar to that of Figure 1, except that fatty acids from titer are plotted on the abscissa. As was the case with cholesterol, no correlation is evident between the concentrations of fatty acids in sera and transudates. A definite proportion exists between the protein and lipid contents of the fluids except in those instances which are marked by circles.

If the normal capillary wall is impermeable to lipoids and protein, injury, which increases its permeability, will permit the passage of both lipoids and proteins. The quantities of these substances which actually escape from the vessels will depend not only upon the degree of permeability to the individual components, but also upon the concentrations of these components in the serum. It has already been pointed out that the influence of the latter factor is not demonstrable in the data as a whole. It does, however, become evident in the points surrounded by circles, which represent instances when the protein in the serum was low in proportion to the lipoids. It is especially striking in the lowest of these points. In this instance the serum proteins were reduced to half the normal concentration, while the fatty acids were increased to about nine times the normal. Under these circumstances the lipid concentration in the fluid might be expected to be, as it proved to be, higher in relation to the protein concentration than it was in the other observations in which the proportions of lipid and protein in the serum were more nearly normal.

In Figure 3, in which lipid phosphorus and protein are compared, there is the same lack of correlation between the concentrations of the former in serum and fluid and the same direct relationship between transudate phosphatides and proteins, with one exception marked with a cross. The significance of this exception must be discounted because the peritoneal fluid, taken at autopsy from a patient with peritonitis, was semi purulent. Some of the lipid phosphorus may, therefore, have come from pus cells and bacteria.

DISCUSSION

It is obvious that the distribution of lipoids to the tissues by the blood stream must involve their passage through the capillary walls, in spite of the demonstrated impermeability of the latter. These experiments throw no light on the means by which this transportation is effected, whether the character of the lipoids is altered (3, 4, 22, 23, 24), or whether the impermeability of the capillaries is merely a facultative characteristic. In most of the standing experiments, during the half hour period, an equilibrium appears to have been reached and main

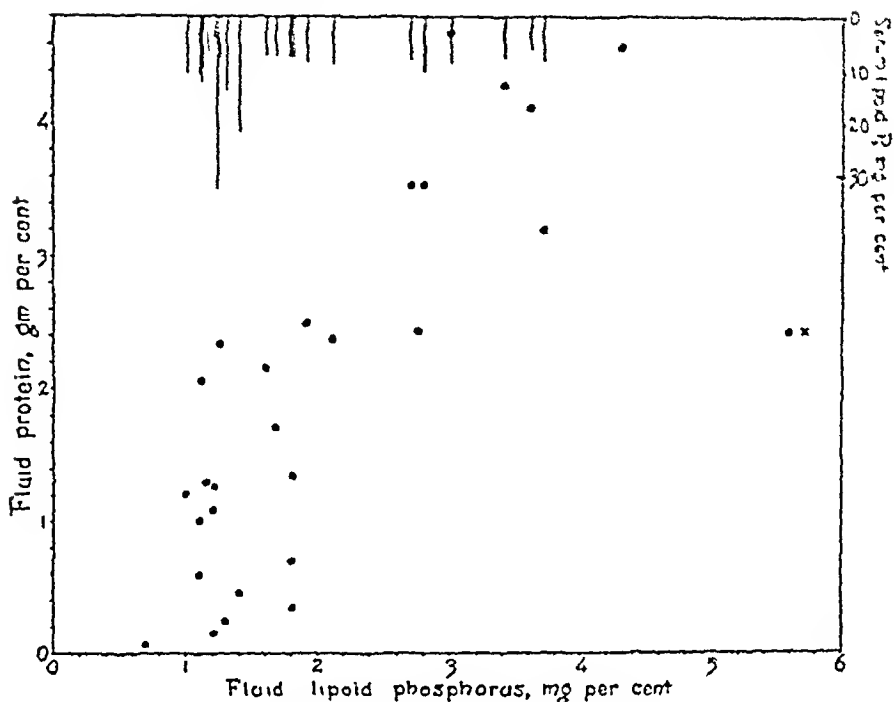


FIG 3 COMPARISON OF LIPOID PHOSPHORUS AND PROTEIN IN TRANSUDATES

The vertical lines above indicate the concentrations of fatty acids in the sera of the patients from whom the transudates were secured. The point marked by a cross represents peritoneal fluid secured *post mortem* from a patient with peritonitis.

tained, in which the concentration of all lipoids, like that of proteins, was influenced only by the hemoconcentration that resulted from the loss of serum ultrafiltrate to the tissues. In two cases fatty acids and phospholipoids seem to have been simultaneously affected by some other influence, presumably connected with the metabolism of fat. That cholesterol was not similarly involved is only one more addition to the great body of evidence that this lipid fraction is not directly concerned with fat metabolism. The problem of the exchange of lipoids between blood stream and tissue cells becomes even more mysterious when, from the studies of transudates, it becomes apparent how little lipid material (8, 14) gains access through normally permeable vessels to the interstitial fluids, which presumably serve as the medium of exchange between blood and cells.

The association between lipoids and proteins (2, 12, 15, 18, 20, 21, 29) in both types of experiment might suggest that the lipoids are restrained by combination with proteins. Such a deduction is not, however, obligatory. Such combinations may exist and the proteins may act as vehicles for a certain amount of lipid material. In the case already

referred to in which protein was reduced and lipid greatly increased in the serum, a greater amount of lipid might have been expected in the transudates if it had been restrained from traversing the walls of the vessels only by its combination with protein.

The exact correlation between the changes of cholesterol and protein in these experiments has a bearing on the nature of the interstitial fluids. Thompson, Thompson and Dailey (25), comparing the volume of the blood, determined by the dye method, with the concentration of proteins in the serum, concluded that the fluid which passed from the blood stream into the tissues of the leg contained no protein. Waterfield (27), in similar experiments, in which he measured the blood volume by the carbon monoxide instead of the dye method, believed that he detected evidence of serum protein loss probably involving only the albumin fraction. More recently Youmans, Wells, Donley and Miller (30) have shown that the protein fractions and total proteins of the serum are all proportionately affected by the hemoconcentration. To these cholesterol can now be added. It is hardly conceivable that if the capillary membranes, under the conditions of these experiments, were permeable enough to permit appreciable quantities of protein to pass out, both fractions and cholesterol would all escape in exactly the same proportions. Certainly such an association is entirely incompatible with Waterfield's conclusions. Krogh, Landis and Turner (13) also found no evidence of loss of serum protein when transudation was induced in the arm by venous obstruction. It becomes harder in the light of these experiments to believe that capillary filtrates produced by venous stasis can contain as much protein as Drinker and Field (6) believe.

If the capillary walls are impermeable to lipoids, the latter may exert an appreciable colloid osmotic pressure in the blood stream and, on this account, have some influence upon the exchange of fluids in the body. Indirect evidence of various kinds indicates that the osmotic pressure of the lipoids is extremely small (1, 7, 28), but more exact data on the subject are greatly to be desired.

CONCLUSIONS

By standing experiments aimed to produce physiological transudation of fluid from the blood stream, and by comparisons of proteins and lipoids in serum and in pathological transudates, evidence has been adduced which indicates that the capillary walls are ordinarily impermeable not only to proteins but also to cholesterol, phosphatides, and compounds of saturated and slightly unsaturated fatty acids.

BIBLIOGRAPHY

1. Adair, G. S., and Robinson, M. E., *Biochem. J.*, 1930, xxiv, 1864. The Analysis of the Osmotic Pressures of the Serum Proteins and the Molecular Weights of Albumins and Globulins.

- 2 Bennhold, H , *Klin Wehnschr* , 1932, vi, 2057 Über Transportprobleme im tierischen Organismus
- 3 Berend, N , *Biochem Ztschr* , 1930, ccxviii, 323 Der Einfluss verschiedener Nahrungsmittel auf die Menge der vielfach ungesättigten Säuren im Blute
- 4 Berend, N , *Biochem Ztschr* , 1932 ccxvii, 117 Über die Rolle der stark ungesättigten Fettsäuren im Organismus
- 5 Bruckman, F S , D Esopo, L M , and Peters, J P , *J Clin Invest* , 1930, viii, 577 The Plasma Proteins in Relation to Blood Hydration IV Malnutrition and the Serum Proteins
- 6 Drinker, C K , and Field, M E , *Lymphatics, Lymph and Tissue Fluid* Williams and Wilkins Company, 1933, Baltimore
- 7 Fishberg, E H , *J Biol Chem* , 1929, lxxvi, 205 The Relations of the Serum Proteins and Lipids to the Osmotic Pressure
- 8 Foord, A G , Youngberg, G E , and Wetmore, V , *J Lab and Clin Med* , 1929, xv, 417 The Chemistry and Cytology of Serous Fluids
- 9 Gáal, A M , *Ztschr f d ges exp Med* , 1930, lxxi, 690 Untersuchungen über Cholesterinstoffwechsel
- 10 Gardner, J A , *J Laryng and Otol* , 1932, xlvii, 395 Cholesterol Metabolism
- 11 Gardner, J A , and Gainsborough, H , *Biochem J* , 1925, xv, 667 Cholesterol Secretion in the Urine Part I
- 12 Gardner, J A , and Gainsborough, H , *Biochem J* , 1927, xvi, 141 Studies on the Cholesterol Content of Normal Human Plasma Part II The Attraction of the Proteins of Plasma for Sterols
- 13 Krogh, A , Landis, E M , and Turner, A H , *J Clin Invest* , 1932, vi, 63 The Movement of Fluid Through the Human Capillary Wall in Relation to Venous Pressure and to the Colloid Osmotic Pressure of the Blood
- 14 Macheboeuf, M A , and Fethke, N , *Bull Soc chim biol* , 1932, xv, 507 Recherches sur les lipides et les protéides des liquides d'épanchements non purulents des séreuses
- 15 Macheboeuf, M A , and Sandor, G , *Bull Soc chim biol* , 1931, xiii, 745 Recherches sur la nature et la stabilité des liaisons protéides-lipides du sérum sanguin Étude de l'extraction des lipides par l'éther en présence d'alcool
- 16 Man, E B , and Gildea, E F , *J Biol Chem* , 1932, xcix, 43 A Modification of the Stoddard and Drury Titrimetric Method for the Determination of the Fatty Acids in Blood Serum
- 17 Man, E B , and Peters, J P , *J Biol Chem* , 1933 (in press) Gravimetric Determination of Serum Cholesterol Adapted to the Man and Gildea Fatty Acid Method, with a Note on the Estimation of Lipoid Phosphorus
- 18 Merklen, Pr , Le Breton, E , and Adnot, A , *Compt rend Acad d sc* , 1931, ccxi, 1053 Influence des lipides du sérum sur la précipitation et le dosage des globulines sériques
- 19 Ni, Tsang-G , and Rehberg P B , *J Physiol* , 1931, lxxi, 331 On the Influence of Posture on Kidney Function
- 20 Reiner, L , *Biochem Ztschr* , 1927, cxvi, 158 Zur Kenntnis des Serum-globulins
- 21 Sørensen, S P L , *Compt rend d trav du lab Carlsberg* , 1930, xii, 1 The Constitution of Soluble Proteins
- 22 Tangel, H , *Biochem Ztschr* , 1930, ccxvi, 180 Mikromethode zur Bestimmung von mehrfach ungesättigten Fettsäuren

- 23 Tangl, H, and Berend, N, *Biochem Ztschr*, 1930, ccxx, 234 Die Fettresorption durch die Desaturation der Fettsäuren
- 24 Tangl, H, and Berend N, *Biochem Ztschr*, 1931, ccxxxii, 181 Weitere Versuche über die Fettresorption durch die Desaturation der Fettsäuren
- 25 Thompson W O, Thompson P K, and Dailey, M E, *J Clin Invest*, 1927-28 v, 573 The Effect of Posture upon the Composition and Volume of the Blood in Man
- 26 Turner, M E, and Gibson, R B, *J Clin Invest* 1932, xi, 735 A Study of the Protein lipid Combinations in Blood and Body Fluids I Normal Human and Dog Plasma and Horse Serum
- 27 Waterfield, R L, *J Physiol*, 1931, lxxii, 110 The Effects of Posture on the Circulating Blood Volume
- 28 Wells, H S, *Am J Physiol*, 1932, ci, 409 Some Criteria of Accuracy for the Measurement of the Osmotic Pressure of Colloids in Biological Fluids
- 29 Went, S, and Goreczky, L, *Biochem Ztschr*, 1931, ccxxxix, 441 Die Verteilung der Serumphosphatide und des Cholesterins in durch Filtration durch grossporige Kollodiumfilter gewonnenen Serumultrafiltraten
- 30 Youmans, J B, Wells, H S Donley, D, and Miller, D G, *J Clin Invest, Proc*, 1933, xii, 965 The Effects of Posture (Standing) on the Serum Protein Concentration and Colloid Osmotic Pressure of Blood from the Foot in Relation to the Formation of Edema

THE CLINICAL SIGNIFICANCE OF THE ERYTHROCYTIC SEDIMENTATION TEST IN RHEUMATOID ARTHRITIS¹

BY WENDELL J STAINSBY AND EDITH E NICHOLLS

(From the New York Hospital and the Department of Medicine of Cornell University Medical College New York City)

(Received for publication June 28, 1933)

Several investigators (1, 2, 3, 4) have called attention to the increased sedimentation rate of the red blood cells in patients with rheumatoid arthritis. In order to ascertain the practical importance of this phenomenon, an extensive study was undertaken which continued for three years. During this period, routine sedimentation tests were carried out on all patients with rheumatoid arthritis on admission to the arthritis department of the Cornell Clinic. When possible, the test was repeated at varying intervals as long as a patient was under observation.

Altogether 597 cases were included in this study, and the purpose of the following paper is to present an analysis of our findings.

TECHNIC

The technic of the sedimentation test was similar in all respects to that of Rourke and Ernstene (5) and need not be described here. The method of these investigators is particularly applicable in rheumatoid arthritis because of its correction for anemia which is a common though variable factor in this disease. Heparin was used as an anticoagulant throughout the study and appeared to have no appreciable effect on the sedimentation rate. The test was recorded as the corrected sedimentation index (C S I) and represents the rate of fall of the erythrocytes in millimeters per minute, after correcting for anemia. Any result up to 0.4 was considered normal. Unless otherwise stated, all sedimentation results reported refer to those at the time of the patient's first visit to the Clinic.

Relation of the corrected sedimentation index to the activity of the disease

In this report, the term "activity of the disease" refers to the severity of the infectious process. It must be recognized that this activity does not always parallel the degree of joint involvement as illustrated by "burned out" cases of arthritis in which swelling and deformity of the joints are present but the disease process is entirely quiescent.

¹ This study was carried on with the technical assistance of Edith L. Ross, Edna H. Lindsay, Edith M. Kirkpatrick, and Elnora B. Carmichael.

In patients with rheumatoid arthritis, the activity, or severity, of the disease is often difficult to determine clinically. It is roughly estimated from the amount of pain and tenderness in the joints, from the character rather than the degree of the swelling, and from the tendency of the process to migrate from joint to joint. However, temperamental factors, which vary considerably in different patients and at different times in the same individual, frequently give rise to an entirely erroneous impression concerning the activity of the disease. Weather and occupational conditions, likewise, play more or less important rôles in confusing the clinical picture. In order to determine whether the difficulties caused by these variants could be eliminated by use of the sedimentation test, the results of this method when applied to a selected group of intelligent, mentally stable, and cooperative patients were analyzed. The following protocols illustrate our findings.

Case 1 H W, female, aged 45, admitted to the clinic March 16, 1928, gave a history of polyarthritis of six years duration. At the time of admission, pain and stiffness of the joints were slight, and the patient was pursuing her usual occupation as a typist. Physical examination revealed nothing of importance except in the joints—there was a slight but definite fusiform swelling of the fingers, with a low degree of swelling of the left knee and both ankles. A diagnosis of mild rheumatoid arthritis was made. During the succeeding three years, this patient was under our observation and throughout this period there was remarkably little change in her symptoms and physical condition. The following results of the corrected sedimentation tests, taken over part of that time, closely paralleled the clinical impression: January 1, 1931, 0.6, April 29, 1931, 0.55, September 25, 1931, 0.7, October 16, 1931, 0.6, February 29, 1932, 0.6.

Case 2 J Mc, male, aged 20, admitted to the clinic on January 16, 1931, gave a history of polyarthritis of two years duration and complained of considerable pain at the time of admission. Physical findings were unimportant except for the joint condition—the left hand and right knee were considerably swollen and slightly tender. During the 15-month period that the patient was under observation, the feet, heels, shoulders, elbows, and right hand gradually became involved. He consistently complained of much pain, and tenderness was frequently noted. A diagnosis of rheumatoid arthritis was made. It was considered a very active and rapidly progressing case, without any remission during the period of observation. With this patient, the sedimentation rates closely paralleled the clinical impression, being high each time the test was made. The results of these tests were as follows: January 16, 1931, 1.7, May 1, 1931, 1.2, October 30, 1931, 1.6, November 25, 1931, 1.3, and March 16, 1932, 0.75.

Case 3 N DeV, male, aged 46, was admitted to the clinic August 22, 1930, with a history of a rapidly progressing polyarthritis of nine months duration. Physical examination revealed the typical fusiform swelling of the fingers, swollen knees and ankles, and stiffness of shoulders and hips. Several of the joints were tender. The remainder of the examination was irrelevant, except for the presence of a duodenal ulcer that caused no symptoms while the patient was under observation. He showed no improvement while attending the clinic, and was admitted to the hospital January 2, 1931. A corrected sedi-

mentation test, carried out on December 29, 1930, a few days before admission, was 1 23 With rest in bed the patient made rapid progress toward recovery, and was discharged from the hospital on February 28 1931, as a cured case At that time he was entirely free from symptoms There was, however, a small amount of swelling still remaining in the fingers and wrists, but this was considered as due to the scarring resulting from the disease, rather than as representing an active process The corrected sedimentation tests during his stay in the hospital closely paralleled his clinical improvement, being 1 2 on January 2 1931 0 7 on February 7, 1931 and 0 4 on February 25, 1931—the figure recognized as normal in a corrected sedimentation test Following his discharge from the hospital, the patient returned to work On resuming his occupation, there was a marked return of the arthritic process On May 19, 1931, his clinical condition approximated that on admission to the hospital four months previously, and his corrected sedimentation test was 1 6 During the succeeding year this case was followed in the out patient department, but there was no appreciable change noted On April 29, 1932, when last seen, his arthritis was unimproved and his corrected sedimentation test was 1 9

Case 4 M O, female, aged 40, was admitted to the clinic March 14, 1930, with a three year history of pain and intermittent swelling in fingers and ankles Symptoms had never been severe, and she had been able to continue her household activities Physical findings were unimportant except in respect to the joints There was questionable swelling of the proximal interphalangeal joints and slight puffiness around the ankles A diagnosis of mild rheumatoid arthritis was made During the succeeding year she was observed in the clinic at irregular intervals, with little if any evidence of arthritis On February 3, 1931, the date of her first test the corrected sedimentation index was 0 2

Beginning in March 1931, her arthritis gradually grew worse, and the fingers took on a marked fusiform swelling In succession the knees, wrists, elbows, and shoulders became swollen and painful There were aching pains in practically every joint in the body The sedimentation test coincided perfectly with the change in her clinical condition, the index being 0 9 on April 15, 1931 and 1 3 on April 23, 1931 On May 11 1931 she again attended the clinic At that time she was barely able to walk and her sedimentation index was 1 3 On May 23 of the same year, she made her last visit to the clinic and her index at that time was 1 1 Following this observation, she reported by telephone that she was unable to leave her bed

Case 5 J M male, aged 24 admitted to the clinic on December 20, 1930, gave a history of a rapidly progressing polyarthritis of ten weeks duration Physical examination showed little of importance except for the joint condition There was fusiform swelling of several fingers, with swollen and tender knees and ankles A diagnosis of rheumatoid arthritis was made and the condition was so severe that the patient was admitted immediately to the hospital The sedimentation index on December 20, 1930, was 2 0 On the day of admission, the patient's temperature was 99 5° F, and was normal thereafter With rest in bed and hospital care the arthritis rapidly improved from the day of admission On February 13, 1931, the swelling had completely disappeared, and on February 25, twelve days later, the patient was discharged as free from signs and symptoms of the disease The clinical change was correctly and immediately registered in his sedimentation tests On December 29, 1930, the index was 0 65 January 13, 1931, it was 0 4 on February 7 and 25 of the same year it was 0 1 and 0 2, respectively

The first protocol is that of a patient running a mild course of arthritis throughout observation. Her sedimentation indices recorded at varying intervals during this time were remarkably consistent with the clinical findings, being slightly above normal each time the test was made. A similar relation between the sedimentation rate and the clinical findings was revealed in the second protocol, although the second patient had a severe process, and his sedimentation indices were consistently high.

Protocols 3, 4, and 5 are of patients whose clinical picture varied markedly during observation. The third patient had a complete remission, the fourth developed a severe form of the disease from a very mild one, while the fifth recovered completely from a very active arthritis. The sedimentation indices in each of these cases correctly and immediately registered the changes in the patient's physical condition.

In the other patients of our series the relationship between the clinical picture and the sedimentation rate is similar to that in the five protocols given. Regardless of the fundamental factors underlying the sedimentation phenomenon, it is apparent that the erythrocytic sedimentation index parallels closely the severity, or activity, of the arthritic process.

Relation of corrected sedimentation index to the degree of joint involvement

In the preceding section of our report, it was shown that the corrected sedimentation index is directly related to the activity, or severity, of the arthritic process. In the present section, a comparison of this index with the degree of joint involvement is considered. A differentiation between the activity, or severity, of the disease and the degree of joint involvement must be made, as it is recognized clinically that some patients suffering from a severe form of the disease have considerable pain, malaise, and even disability, with little change in the joints that may be detected by physical examination, whereas a patient may have marked swelling of the joints with deformity and ankylosis without the symptoms named above.

In Table 1, the 597 patients studied are tabulated according to degree

TABLE 1

Relation of corrected sedimentation index to degree of joint involvement

Type of case	Number of cases	Lowest C.S.I.	Highest C.S.I.	Average C.S.I.
Without joint swelling	308	0.1	2.0	0.64
With joint swelling	232	0.1	2.1	0.85
With swelling, deformities and ankylosis	57	0.3	2.6	1.11
Total	597			

of joint involvement Three hundred and eight of these had no appreciable joint changes at the time of examination, their sedimentation indices ranged from 0.1 to 2.0, with an average of 0.64 Two hundred and thirty-two of them had joint swelling without ankylosis, or deformities other than swelling their sedimentation indices varied from 0.1 to 2.1, with an average of 0.85 Fifty seven of them were in the more advanced stages of the disease with deformities such as ulnar deviation and ankylosis, the sedimentation indices of this group ranged from 0.3 to 2.6, with an average of 1.11

From these results it is evident that, on the average, the greater the degree of swelling, deformity, and ankylosis, the higher the sedimentation rate, and, therefore, the greater the activity, or severity, of the disease, but as far as the individual patient is concerned, this deduction cannot always be made

Relation of the corrected sedimentation index to the age of the patient

In Table 2, the corrected sedimentation indices of the 597 cases are arranged according to the age of the patient In this comparison, an interesting though unexpected relation was revealed In patients in the third and fourth decades of life, a similar average sedimentation index was obtained but in those 40 years of age or over the average sedimentation rate showed a gradual increase all the way to the last group composed of patients 60 years of age or older

TABLE 2

Relation of corrected sedimentation index to age of patient

Age of patient	Number of cases	Average C.S.I.
Under 30 years	69	0.69
From 30 to 39 years	122	0.68
From 40 to 49 years	181	0.73
From 50 to 59 years	170	0.83
60 years or over	55	0.96
Total	597	

The explanation of this gradual increase in the sedimentation rate with advancing years is not altogether clear In the first place, it would seem probable that as patients grow older they tend to develop disease conditions other than arthritis which also affect the sedimentation rate Our records indicate, however, that this factor plays no important rôle in the phenomenon, as no appreciable increase in disease unrelated to arthritis was detected in the older groups of our series In the second place, it was observed that each succeeding decade is augmented by an increasing proportion of patients with severe arthritis who failed to recover from the disease earlier in life, as evidenced by an increase of the average disease duration with each succeeding age period It seems

reasonable to conclude, therefore, that the increase in the average sedimentation rate with each succeeding age period is due primarily to the increase in the number of patients with severe arthritis, although other factors may play minor rôles in the results

Relation of corrected sedimentation index to season of year

Clinically it is generally recognized that in temperate climates patients with rheumatoid arthritis are usually at their worst in cold weather and at their best in hot weather. In order to determine whether this impression is confirmed by the sedimentation test, the indices of patients followed through the various seasons were analyzed. All patients on whom tests had been made in at least three seasons were included. With those having more than one test during any one seasonal period, the average was considered as that patient's index for that season. In the study, "Winter" covers the period from January 1 to May 31, "Spring" from April 1 to June 30, "Summer" from July 1 to September 30, and "Autumn" from October 1 to December 31.

TABLE 3

Relation of corrected sedimentation index to season of year

Season of year	Number of indices	Average CSI
Winter	58	0.865
Spring	62	0.840
Summer	38	0.799
Autumn	55	0.846

Altogether 69 patients were studied for seasonal variation. On these 69 patients, 58 tests were recorded in the winter, 62, in the spring, 38, in the summer, and 55, in the autumn. In Table 3, the average corrected sedimentation index for each of these recorded groups is presented. The highest figure occurred in winter, and the lowest, in summer, while spring and autumn gave intermediate results. The number of cases studied and the differences between the seasonal averages were too small to warrant definite conclusions. However, it is strongly suggestive that seasonal variations in the average sedimentation indices do occur, and as this test is an index of the activity, or severity, of the arthritis, that patients, on the average, are worse in the winter and better in the summer.

Relation of the corrected sedimentation index to the duration of the disease

The records of the patients under observation were studied again to discover whether any relationship existed between the corrected sedimentation index and the duration of the disease. In the individual records little such relationship was found—some patients having had the disease for only a few weeks gave high sedimentation rates, while others having had it for many years gave a low sedimentation rate, and

vice versa In studying the average rates of sedimentation for various disease duration periods, however, it was found that there was a distinct tendency for these rates to increase as the duration of the disease increased (Table 4) From these results we conclude that patients having rheumatoid arthritis for a long period tend to have a more severe form of the disease than those having it for a short period, but that great individual variation exists

TABLE 4

Relation of corrected sedimentation index to duration of disease

Duration of disease	Number of cases	Average C.S.I
One month or under	9	0.70
Over 1 month to 3 months	50	0.71
Over 3 months to 6 months	63	0.70
Over 6 months to 1 year	87	0.77
Over 1 year to 3 years	120	0.76
Over 3 years to 5 years	74	0.79
Over 5 years	194	0.79
	<hr/> 597	

Relation of the corrected sedimentation index to the streptococcus agglutination test

In previous articles (6, 7), the authors showed that a high percentage of patients with rheumatoid arthritis gave a strong agglutination reaction with a biologically specific type of hemolytic streptococcus which, for convenience, they designated as "typical strain" This reaction appeared to be a true immunological response to a bacterial invader The question naturally arises as to what relationship, if any, exists between this agglutination reaction and the erythrocytic sedimentation rate An examination of the records of the cases in our series indicated that there was none whatever In patients with rheumatoid arthritis, the agglutinins are slow in developing and do not reach their maximum titer until about 6 months, on an average, has elapsed Following recovery of the patients, the agglutinins gradually diminish, but do not disappear, usually, until the patient has been free from the disease for several months, and sometimes years The sedimentation rate, on the other hand, is directly related to the activity, or severity, of the disease process, and changes directly with variations in this activity The difference in these two tests may be appreciated readily from a study of Figure 1, which is a graphic record of the agglutination and sedimentation tests of Case 5, previously described, and is typical of other similar records

DISCUSSION AND SUMMARY

The erythrocytic sedimentation test was performed on five hundred and ninety seven patients with rheumatoid arthritis in order to determine its significance in this disease

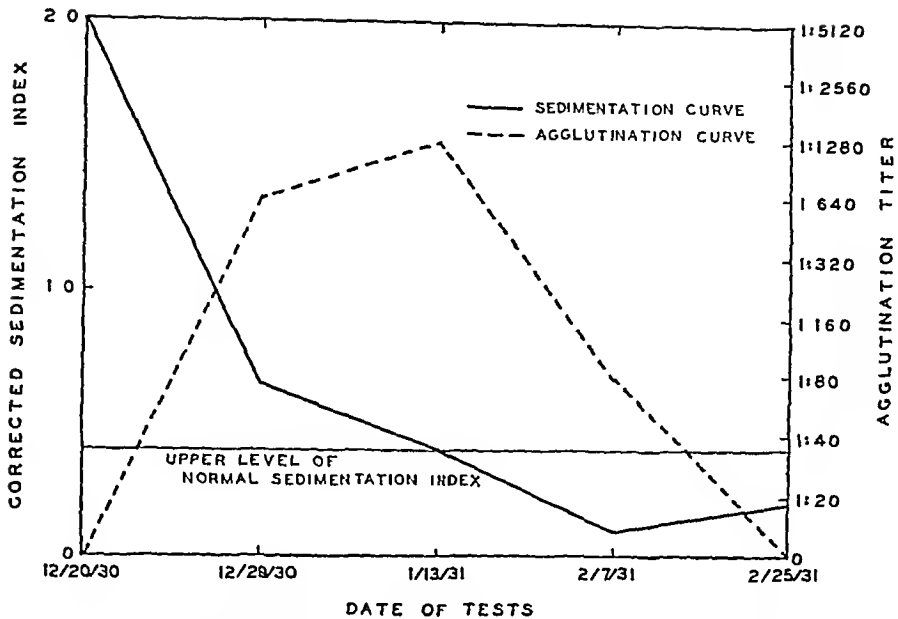


FIG 1 A COMPARISON OF THE RESULTS OF THE SEDIMENTATION AND AGGLUTINATION TESTS OF CASE 5

The results of the investigation indicate that the corrected sedimentation index is a reliable criterion of the activity, or severity, of the arthritic process at the time of testing, and that any fundamental change in the clinical condition produces immediate corresponding change in the sedimentation rate

Patients with a greater degree of joint involvement and a longer disease-duration have higher sedimentation rates, on the average, than those with less joint involvement and shorter disease-duration. Considerable variation occurs, however, in individual cases

The observation that the average sedimentation rate progressively increases with advancing age periods is of interest. This phenomenon appears to be due primarily to the increasing accumulation of patients with severe arthritis in whom the disease began at some earlier age

From a study of seasonal variations in the sedimentation rate over a long period, suggestive evidence was deduced that, on the average, the rate was higher in winter than in summer, while spring and autumn occupied intermediate positions. Further work is necessary in order to confirm this impression

No relationship was found between the sedimentation rate and the streptococcus agglutination reaction

With the above information at hand, it seems justifiable to discuss the practical importance of this test. In the past, physicians treating arthritis have been greatly handicapped by lack of means for estimating

the results of their therapy The sedimentation test appears to supply this widely felt need, as it is a reliable measuring rod of the activity, or severity, of the arthritic process By repeating this test at regular intervals, the progress of the patient may be determined

Rheumatoid arthritis has attracted more than its share of ill conceived treatments The sedimentation test provides a ready aid for correctly estimating the value of such procedures

BIBLIOGRAPHY

- 1 Kahlmeter, G, *Klin Wchnschr*, 1926, v, 889 Über die Bedeutung der Fåhræusschen senkungsreaktion bei akuten und chronischen Arthritiden
- 2 Dawson, M H, Sia, R H P and Boots, R H, *J Lab and Clin Med*, 1930, xv, 1065 The Differential Diagnosis of Rheumatoid and Oste arthritis The Sedimentation Reaction and Its Value
- 3 Weiss, Arthur, *Am J M Sc*, 1931, clxxx, 379 The Prognostic Value of the Sedimentation Rate in Arthritis A Modification of the Technique
- 4 Oppel, T W, Myers W K, and Keefer C S, *J Clin Invest* 1933, xii, 291 The Sedimentation Rate of the Red Blood Cells in Various Types of Arthritis
- 5 Rourke M D, and Ernstene, A Carlton *J Clin Invest* 1930, viii, 545 A Method for Correcting the Erythrocyte Sedimentation Rate for Variations in the Cell Volume Percentage of Blood
- 6 Nicholls, E E, and Stainsby, W J, *J Clin Invest*, 1931, x, 323 Streptococcal Agglutinins in Chronic Infectious Arthritis
- 7 Nicholls, E E, and Stainsby, W J, *J Clin Invest* 1933, xii, 505 Further Studies on the Agglutination Reaction in Chronic Arthritis

THE OXYGENATION OF CONCENTRATED VERSUS NORMAL BLOODS

By GEO B RAY, C I THOMAS AND J E STRONG¹

(From the Department of Physiology Western Reserve University School of Medicine,
Cleveland)

(Received for publication June 28 1933)

While the physiological activity of normal or dilute bloods represents one of the most widely studied fields of investigation, the problems presented by high concentrations of hemoglobin have not received the attention they merit. As far as the writers have been able to determine comparatively little attention has been paid to the ability of polycythemic bloods to take up oxygen under normal conditions. Barcroft and Murray (1) compared the effect of concentrating blood by centrifugating and removing part of the plasma. For a given O₂ tension they found a higher degree of saturation in the concentrated blood than in normal blood which in turn took up more O₂ than did a sample diluted with the plasma which had been taken from the concentrated blood. This variation was considered secondary to a disturbance in the carbon-dioxide equilibrium. Richards and Strauss (2) found no difference between the dissociation curve of the blood of a polycythemic patient and that of blood from a normal individual.

The experiments reported in this paper fall into two groups the first designed to repeat the tests of the oxygen combining power of concentrated blood *in vitro*, and the second to observe and compare the oxygenation of concentrated blood with that of normal blood in the perfused lung where the experimenter has a maximum control over the factors associated with oxygenation. Such a series of experiments should offer a concrete and definite answer to the question, does a plethora of red cells, *per se*, influence the respiratory function of the blood.

Experiments on the O₂ combining power of concentrated blood

The technique employed in the handling of the blood and gases in these experiments was essentially that described by Austin et al (3). The concentrated blood was obtained by centrifugating blood of normal dogs and pipetting away most of the supernatant plasma. A measured amount of this blood, of known oxygen capacity, was placed in a small tonometer bearing at one end a two way stopcock and at the other a

¹ Crile Research Scholar

tube of fairly large bore. The tubular end was connected to a similar end of a large tonometer of the same type having a capacity of 250 to 300 cc. Care was taken to see that the blood filled the smaller tonometer well into the rubber connection. This connection was then clamped and the pair of tonometers connected with a manifold whereby one could either evacuate the vessel or supply CO_2 -free air, nitrogen, or carbon dioxide. The tonometer was repeatedly evacuated and washed with nitrogen after which sufficient CO_2 -free air was admitted to give the desired oxygen tension, following which the CO_2 tension was adjusted to 40 millimeters. Pressure within the tonometer was then brought to atmospheric by the admission of nitrogen. With the cocks closed the tonometer was removed from the manifold and the clamp between the blood and gas chamber taken off, allowing the blood to flow into the larger chamber. In this manner portions of each sample of blood were brought into equilibrium with gas mixtures of five different oxygen tensions. The tonometers were rotated simultaneously in an air bath at 37°C for thirty minutes. At the end of the period of equilibration the blood was allowed to return to the small chamber and the clamp replaced. The chambers were then disconnected, and samples taken for analysis by forcing the blood into a pipette with mercury pressure. At no time was the blood allowed to come in contact with the room air. The analyses for oxygen content were made with the Van Slyke and Neill constant volume manometric apparatus, oxygen capacity was estimated by means of the spectrophotometer (Ray, Blair and Thomas (4)). The final O_2 tensions existing in the tonometers were corrected for oxygen lost from the blood and for the effect of temperature change.

The results of a characteristic series of experiments are given in Figure 1. The open circles represent the results found in the normal blood and the black those for the polycythemic blood. Both samples were from the same original blood in order to avoid any chemical factors which might influence the results. These data show beyond any doubt that the oxygen-combining power of the two bloods is identical, under the conditions of the experiments. In fact the distribution of the two sets of points coincides quite as well as would points secured from two samples of the same concentration. The variation of any point from the mean curve is within the experimental error of the methods employed.

There is a point of distinction which can not be demonstrated in the graph, but was definitely apparent during the course of the experiment. The time required for the concentrated blood to reach an equilibrium was markedly longer than that needed for normal blood. While the normal sample showed its maximal color change in five to ten minutes after the start of equilibration the concentrated needed almost double the time for the same change. This was a natural sequence of the concentration of the blood since diffusion is inversely proportional to viscosity.

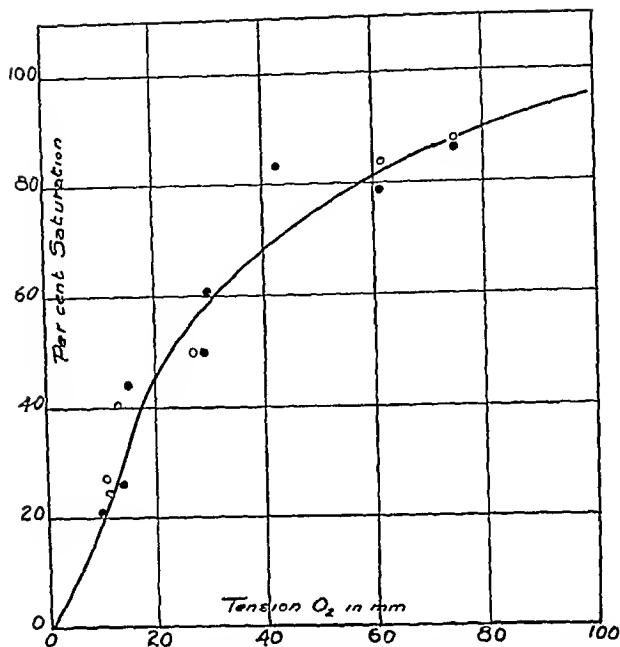


FIG 1 DISSOCIATION CURVE OF NORMAL AND POLYCYTHEMIC BLOODS

The open circles represent blood of normal hemoglobin concentration and the black, polycythemic blood

and also the amount of gas moved was greatly increased. The effect of both factors will be discussed in detail in a later section.

Experiments with the perfused lung

The preceding experiments give no tangible evidence of a difference between the oxygen combining power of the two types of blood. Obviously if a difference does exist it must be sought in other directions. We turned, therefore, to the perfused lung. These experiments were planned to determine if a normal or approximately normal oxygenating apparatus was capable of working with its usual efficiency when the number of cells passing through the tissue per unit of time was increased. That is, if the rate of flow of hemoglobin through the lung is doubled can the respiratory conditions be so adapted as to produce a normal degree of oxygenation? The importance of blood flow through a tissue

has often been stressed, but in this case it is, perhaps, preferable that attention be directed to the amount of hemoglobin as the essential factor

In order to study this effect we were forced to use a perfused lung. Preliminary experiments on intact anesthetized dogs gave uncertain results. The results reported in this section were made on the isolated lungs of cats with the conditions of ventilation, blood flow, etc., made to simulate as closely as possible, normal conditions, while allowing full control over every variable. It was thus possible to study the respiratory function without the interference of the generalized compensatory mechanisms which were likely to be brought into play in the intact animal in response to a sudden massive injection of corpuscular cream.

A diagram of the apparatus used in perfusing the lung is given in Figure 2. The lungs (1) and heart were removed as rapidly as possible from the anesthetized cat. In order to prevent clot formation during this process the animal was previously injected with heparin. A cannula (2) was inserted into the pulmonary artery by way of the right ventricle. The tracheal cannula (3) was tied in. The outflow cannula (4) was inserted into the left auricle by way of the ventricle and the whole preparation placed in the jar (5) which served as a thorax. The metal plate (6) was clamped in place on a vaselined rubber ring, making an air-tight seal. The basic negative pressure was controlled by connecting tube (8) to a vacuum and expanding the lungs to approximately their normal size. This pressure was controlled by a manometer. The fluctuating negative pressure was produced by a pump (7) having a variable stroke and speed. A tambour connected at (9) recorded these fluctuations of pressure and so, upon calibration, served as a measure of the tidal air volume. This estimation was checked by a water manometer connected at (10) to the tracheal tube. The main branch of the tracheal tube went to a pair of mercury valves (11) of the type described by Bailey (5), which allowed passage of air with a minimum of resistance.

For ventilating the lung atmospheric air enriched with approximately 5 per cent carbon dioxide was used, in order to maintain the acid-base relationships of the blood. This was stored in a 100 liter spirometer connected at (12) from which it was drawn into a smaller graduated spirometer (13) for use. The composition of the stored gas was checked from time to time to detect changes in O_2 and CO_2 content.

The blood used in these experiments was citrated ox blood. It is unfortunate that blood of another species had to be used, but the difficulties associated with collecting a sufficient amount of fresh cats' blood were insurmountable. Careful preliminary experiments were carried out to detect any indication of harmful reactions resulting from the above combination. Even after long periods of perfusion no deleterious effect could be seen other than the appearance of some edema which might have been expected, regardless of the nature of the perfusion fluid.

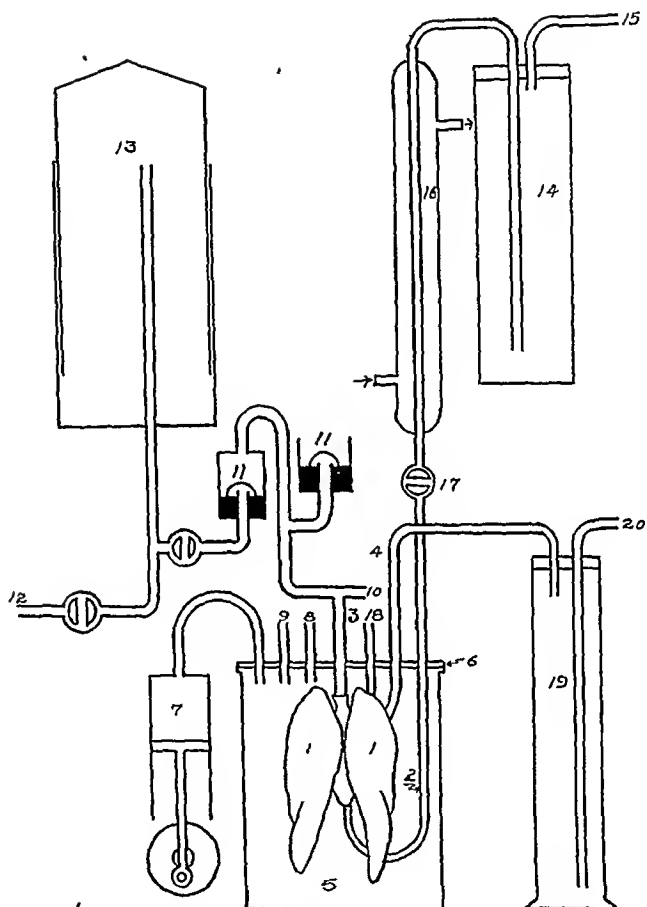


FIG 2 APPARATUS USED IN PERFUSING THE LUNG
Explanation of the figure is given in the text.

The fact that continued perfusions caused no striking changes in blood flow or ventilation was deemed evidence of the nontoxicity of the blood

The blood to be used in any particular series of experiments was divided in advance into two portions. One, the larger, was centrifugated and most of the supernatant plasma removed. Just previous to the experiment the sample to be used at that time was placed in a large bottle tightly stoppered with a two-hole rubber stopper, one opening of which was connected to a manifold by means of which vacuum, nitrogen and carbon dioxide could be controlled. The other opening of the bottle was tightly closed. The bottle was then evacuated and the resulting foam broken by the admission of nitrogen. This process was repeated until a vacuum produced no foam, which was taken as an indication that reduction was practically complete. In the case of concentrated bloods the process was, of course, much slower, but was accelerated by saturating the blood with carbon dioxide. The experiments might be criticized as unphysiological since blood of such low O_2 content was used, but it must be remembered that it was desired to test the ability of the lung to allow access of oxygen to the blood. Unless some common base line were adopted this estimation could not be made under exactly comparable conditions. The reduced bloods were kept under an atmosphere of nitrogen until they were ready to be introduced into the perfusion apparatus. At this time the sample was forced into the storage bottle of the apparatus (14) by pressure from a hydrogen tank and was kept under an atmosphere of this gas during the course of the experiment. The storage bottle was connected to an 18 liter bottle connected at (15), which in turn was connected to a constant water pressure. The blood was forced from the storage bottle through a condenser (16) to bring it to the proper temperature, and thence to the pulmonary artery. The pressure in this artery was adjusted by means of the stopcock (17). A record of the pulmonary arterial pressure changes was recorded by a mercury manometer connected at (18). The outflowing blood was caught in a 500 cc graduate (19) which served to measure the total blood flow while the rate of flow was computed from a tambour connected at (20).

Perhaps the feature to be most carefully avoided was the possibility of edema. In the preliminary experiments in which perfusion was carried out over a long period of time, edema was obviously present. In the course of these experiments certain signs were discovered whereby it was possible to recognize this condition at its onset and furthermore to estimate the duration of time that an experiment could be carried on without vitiating the results. Early edema was manifested by an increase in ventilation pressure and a decrease in venous pressure. No experiments in which these signs appeared are reported in this paper. In all experiments the period of perfusion was made as brief as was consistent with securing an adequate series of samples.

The analyses for the degree of saturation were made on the Van Slyke apparatus, while the oxygen capacity was estimated from the hemoglobin concentration determined by means of the spectrophotometer. Both arterial and venous samples were taken as nearly simultaneously as possible for each reading. Any variation in hemoglobin content served as a check on fluid loss, and hence edema. Obviously care had to be exerted that the storage bottle of blood was constantly agitated in order to avoid sedimentation. In the first experiments we attempted to study both normal and polycythemic bloods in the same lung, but the period of perfusion extended over much too long a time and the practice had to be discontinued in favor of individual experiments for each type of blood.

The general nature of the data obtained by this procedure may be illustrated in tabular form by a protocol of a pair of companion experiments.

It will be noted that the degree of saturation of the venous blood in the case of the concentrated sample dropped markedly in the later

TABLE I
Protocol of a typical pair of experiments

Experiment	Time	Blood pressure		Intrathoracic pressure		Arterial blood			Venous blood		
		Arterial	Venous	Inspiration	Expiration	Oxygen capacity	Oxygen content	Per cent saturation	Oxygen capacity	Oxygen content	Per cent saturation
A*	minutes	mm Hg	mm H ₂ O	mm H ₂ O	mm H ₂ O	volumes per cent	volumes per cent		volumes per cent	volumes per cent	
	1 25	10	10	- 50	-20	15 34	4 32	28	14 74	14 83	100 0
	3 60	20	25	- 40	-10	15 27	4 08	26 8	15 14	15 13	100 0
	4 80	10	25	-100	-50	15 42	4 18	27 25	15 42	14 83	96 0
	7 50	40	45	- 60	-30	lost	4 08		15 42	14 90	96 5
B†	1 00	23	24	- 80	-40	39 25	1 42	3 60	37 59	26 82	71 4
	3 20	23	24	-110	-40	40 59	1 42	3 50	37 37	24 58	66 0
	6 50	23	24	-110	-40	39 39	1 42	3 60	38 83	21 88	56 3

* Experiment A Tidal air, 28 cc per respiration Rate of respiration, 40 per minute Lung capacity, 196 cc Average rate of blood flow, 35 cc per minute

† Experiment B Tidal air, 30 cc per respiration Rate of respiration 20 per minute Lung capacity, 166 cc Average rate of blood flow, 40 cc, per minute

readings As far as we could determine this was not due to any change in the lung tissue but rather to a continually diminishing tension of oxygen in the alveolar air resulting from the demands of the concentrated blood Spectrophotometric evidence proved, in addition, that the hemoglobin had retained its ability to combine with oxygen The slight variations noted in oxygen capacity are without doubt due to sedimentation, which could not be completely controlled

The results are summarized in Figure 3 in which the crosses represent the degree of saturation of the normal blood and the circles that of the concentrated blood It will be noted that in every case the normal blood was oxygenated completely or almost completely, which is exactly what one would predict for the oxygen tensions used and the rate at which the blood was flowing through the lungs The results indicate clearly the efficiency of the perfusion system

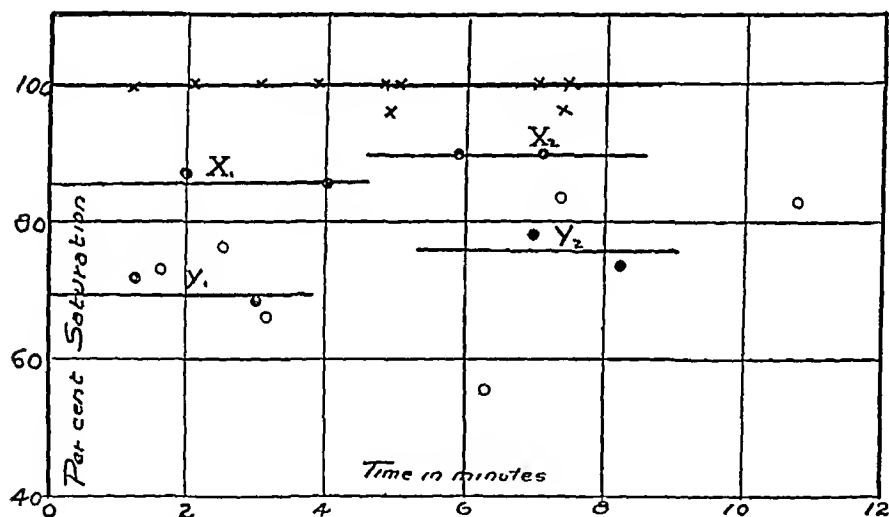


FIG 3 COMPARATIVE RESULTS OF PERFUSING THE LUNGS WITH NORMAL AND POLYCYTHEMIC BLOOD

Crosses represent normal blood, the circles, polycythemic Explanations of curves X_1 , X_2 , Y_1 and Y_2 are given in the text

In the concentrated blood on the other hand, a normal saturation was never attained In spite of the fact that the lungs were to all intents and purposes normal, i e allowed oxygenation of normal blood, it is evident that with the usual ventilation and blood flow the system is incapable of functioning adequately The incapacity of the system may be noted in a very simple manner In two experiments, represented by curves X and Y , an attempt was made to reproduce what would be the normal compensatory reaction of an animal, i e the minute volume of respiration was increased These results are of particular significance

since they show beyond question that the difficulty is not referable to any faults of the apparatus. In the routine experiments ventilation was within the normal resting limits defined by Scott (6) and by Churchill (7), whose observations agreed with our own measurements. It was further noted that hyperpnea, produced by stimulation of the sciatic previous to removing the heart and lung, raised the ventilation to about twice its resting value. Accordingly the minute volume was doubled in order to test the effect of a normal degree of hyperpnea.

The ventilation in the section of the first curve, designated as X_1 , was 700 cc per minute, which is within the normal range. At point X_2 the ventilation was increased to 1330 cc per minute. The increase in saturation is, however, far from proportional to the increase in ventilation, since the change is only from 87 to 90 per cent, which would correspond to a change in alveolar O_2 tension of about 5 millimeters.

The results plotted in curve Y are more striking. The original ventilation, represented by section Y_1 of the curve, was 1200 cc per minute while in the section Y_2 the volume was increased to 2280 cc. per minute. In this case we have started with a moderate hyperpnea and increased the ventilation 190 per cent. In spite of this oxygen increase the saturation of the blood changes only from 70 to 77 per cent, equivalent, approximately, to 9 mm of O . It is obvious that if this represents the proportionality between ventilation and saturation, a tremendous hyperpnea would be required to produce a normal condition in the venous blood. It is conceivable that the oxygen saturation of the blood failed to increase under the unphysiological artificial conditions of these experiments because forced expiration did not have its usual effect, that no matter what the minute volume might be, the alveoli were incompletely aerated and therefore contained air with an unusually low oxygen tension. In order to test the validity of such a criticism the mode of attack was changed. Instead of increasing the amount of air sucked into the lungs at each respiration the amount of oxygen in the air was increased. Under these circumstances the problem of accumulation of nitrogen in the alveoli was eliminated and oxygen saturation depended only upon the rate of diffusion into the blood and subsequent oxygenation. In accordance with this scheme the ventilation gas mixture was changed to 94 per cent oxygen and 6 per cent carbon dioxide. The results proved definitely that even such a concentration of oxygen was barely adequate to approximate normal conditions, with this tension the average saturation was only 91 per cent. This figure indicates that it is about 6 to 7 times as difficult to oxygenate concentrated blood as it is to oxygenate normal blood.

DISCUSSION

Certain definite facts stand out as the result of the above experiments. A. With this method blood of normal concentration is adequately

oxygenated *B* Increasing the number of cells, and consequently the amount of hemoglobin passing through the lung tissue per minute, results in inadequate oxygenation *C* Doubling the amount of ventilation results in only a minor change in the saturation of concentrated blood *D* Only when the oxygen tension of the inspired gas is increased to over six times that of the normal atmosphere does the saturation approximate normal

The question arises as to what physiological reactions are responsible for these differences between bloods of normal and high concentration. The distinction appears to be inherent in the character of the blood and not referable to the conditions of the experiment, since normal blood behaves as it does when it is oxygenated in the physiological manner. The lungs function efficiently as an oxygenating device. In fact, as the blood used was nearly completely reduced the tissue was called upon to do more work than would ordinarily be the case *in vivo*. The use of blood from another species seems to have caused no toxic effects. On the basis of these observations we are inclined, therefore, to dismiss the idea that there was any change in the lung tissue *per se*.

The problem therefore resolves itself into a discussion of what factors in the blood or respiratory mechanism act, either independently or together, to produce the result found. The outstanding factor is perhaps the increased amount of hemoglobin passing through the lungs per minute. The demand for oxygen increases directly as the concentration of hemoglobin. In consequence, oxygen tension in the alveoli and the degree of oxygenation of the blood should diminish as the perfusion progresses. That this is the case is illustrated by the experiment shown in Experiment B, Table I, where the saturation decreased from 71 to 56 per cent during the period of measurement.

This might afford an adequate explanation of the whole phenomenon, were it not for the fact that increased ventilation of the lungs proved insufficient to produce normal oxygenation and also that it was found necessary to increase the oxygen tension to seven times that of normal before full saturation was even approximated. In the case of the experiments in which the ventilation was increased, the change in the degree of saturation was by no means proportional to the increase in the minute volume of respiration. The increased movement of air failed to change the tension of alveolar oxygen to any marked extent. Perhaps more significant is the high pressure necessary to drive a sufficient amount of oxygen into the blood. In other words, the rate of diffusion of oxygen is reduced in the case of the more concentrated blood. This is exactly what Harrop and Heath (8) found in polycythemic individuals. These investigators ascribe this change to alteration of the pulmonary epithelium. This cannot be said to be true in our experiments, inasmuch as the lungs behaved in a normal manner when perfused with blood.

containing a low concentration of cells. It is obvious, therefore, that the reason for the faulty oxygenation of the blood must be sought elsewhere. It will be recalled that in the experiments upon the dissociation curves a longer time was needed for complete equilibration of the concentrated blood. It would seem, therefore, that a certain fraction of the unsaturation can be attributed to the character of the concentrated blood itself.

Still another factor to be considered is the reaction which occurs in the capillaries. In the table cited it will be noted that the amount of blood flowing through the system is approximately the same in both experiments. The viscosity of the concentrated blood is much greater than normal, yet if the second readings of both types of experiments are compared it will be noted that the same pressure produces the same flow. The simplest explanation that can be suggested for this adjustment is dilatation of the smaller vessels. The appearance of the lungs in the two cases certainly substantiated this view. If such a compensation involved the alveolar capillaries a second reason for the impairment of oxygen diffusion is found in the increased distance the gas must pass in order to complete the process of oxygenation. In addition to this effect, the engorgement of the vessels would decrease the alveolar space, i.e., the vital capacity, thereby adding to the factors which promote anoxemia.

SUMMARY

Studies of the dissociation curves of normal and artificial polycythemic bloods, show no difference in the tension of oxygen required to produce a given saturation. When these types of blood are oxygenated by the perfused lung the normal blood becomes completely oxygenated while the concentrated blood is never fully saturated. Increasing the oxygen tension increases the saturation of the concentrated blood.

The difference between the two bloods is ascribed to the greater rate at which hemoglobin in the polycythemic blood passes through the lungs, coupled with a delayed diffusion resulting from capillary dilatation.

BIBLIOGRAPHY

- 1 Barcroft J and Murray C D, *Phil Trans Roy Soc (B)*, 1923 cxi, 469
Some Secondary Effects of Increasing the Proportion of Red Corpuscles in Blood
- 2 Richards, D W Jr and Strauss M L, *J Clin Invest*, 1927, iv, 105
Oxyhemoglobin Dissociation Curves of Whole Blood in Anemia
- 3 Austin J, Cullen, G E, Hastings A B, McLean F C, Peters, J P and Van Slyke D D, *J Biol Chem*, 1922, liv, 121
Studies of Gas and Electrolyte Equilibria in Blood. I. Technique for Collection and Analysis of Blood and for its Saturation with Gas Mixtures of Known Composition
- 4 Ray G B, Blair H A and Thomas, C I, *J Biol Chem*, 1932, xcvi, 63
The Spectrophotometric Determination of Certain Blood Pigments

- 5 Bailey, C V, Proc Soc Exper Biol and Med, 1926, xxiv, 184 A Low Resistance Air Valve
- 6 Scott, R W, Am J Physiol, 1917, xlv, 196 The Effect of the Accumulation of Carbon Dioxide on the Tidal Air and on the H-ion Concentration of the Arterial Blood in the Decerebrate Cat
- 7 Churchill, E D, Am J Physiol, 1928, lxxxi, 274 The Effect of Increased Blood Flow on the Ratio between Oxygen Consumption and Pulmonary Ventilation
- 8 Harrop, G A, Jr, and Heath, E H, J Clin Invest, 1927, iv, 53 Pulmonary Gas Diffusion in Polycythemia Vera

ELECTROLYTES IN HUMAN TISSUE III A COMPARISON OF NORMAL HEARTS WITH HEARTS SHOWING CONGESTIVE HEART FAILURE¹

BY WALTER E WILKINS AND GLENN E CULLEN

(From the Department of Biochemistry Vanderbilt University School of Medicine Nashville Tennessee and the Children's Hospital Research Foundation and the Department of Pediatrics College of Medicine University of Cincinnati Cincinnati)

(Received for publication June 30 1933)

Previous studies from Vanderbilt Medical School (Harrison, Pilcher and Ewing, 1930) (Calhoun, Cullen, Clark, and Harrison, 1930) have shown that the potassium content of heart muscle may be diminished in persons dying with congestive heart failure. The present study was planned to investigate the concentration of other important electrolytes under the same conditions and in normal hearts, i.e., hearts from persons killed in accidents. However, when the pathologic reports were analysed it was found necessary to divide the material into three groups—(1) Normal hearts, (2) an intermediate group of hearts from fatal accidents which show pathologic evidence of cardiac disease, and (3) hearts from persons dying with congestive heart failure. See Table I.

Seventeen hearts, 5 in Group 1, 4 in Group 2, and 8 in Group 3, were analysed for water content, calcium, magnesium, phosphorus, potassium and sodium.

The methods used in this study are those described elsewhere (Cullen and Wilkins, 1933). Special precautions were taken to determine accurately the water content of the tissues. The samples, which were shipped, were sealed in air tight bottles, and any liquid which came out of the tissues during transit was included in the entire sample which was weighed and dried.

RESULTS

The determined values, in most cases based on duplicate analyses, for water, total phosphorus, potassium, sodium, calcium and magnesium are given in terms of wet muscle in Table II. In order to compare the osmotic relationship of the electrolytes in the tissue, the results were calculated to milliequivalents per kilo tissue water, as was done by Van Slyke, Wu and McLean (1923) for blood electrolytes. In this

¹ The experimental data in this report are taken from a thesis submitted by Walter E. Wilkins in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Vanderbilt University.

TABLE I

Individual case data

Case number	Sex	Age	Heart weight	Chief diagnosis and cause of death
		<i>years</i>	<i>grams</i>	
Group 1				
1	M	34		Bullet wound of abdomen, hemorrhage, heart normal
2	M	24	400	Bullet wound of head, heart normal
3	M	25	265	Fracture of skull, ruptured liver and kidney, heart normal
4	M	33	400	Bullet wound through lungs and heart, heart normal
5	M	25		Bullet wound of abdomen, hemorrhage, heart normal
Group 2				
6	M	38	535	Fracture of skull
7	M	31		Bullet wound of abdomen, hemorrhage
8	M	49	450	Lacerations of head, dislocation of hip, (chloroform anesthesia) toxic hepatitis, myocardial hypertrophy, generalized arteriosclerosis
9	M		400	Multiple comminuted fractures of the pelvis, hemorrhage, alcoholic intoxication, generalized arteriosclerosis
Group 3				
10	M	25		Myocardial hypertrophy and dilatation, adhesive pericarditis
11	M	33	770	Myocardial hypertrophy and dilatation, syphilitic aortitis and insufficiency
12	F	23	450	Myocardial hypertrophy and dilatation, syphilitic myocarditis
13	M	72	400	Myocardial hypertrophy, generalized arteriosclerosis
14	M	40	860	Myocardial hypertrophy and dilatation, generalized arteriosclerosis
15	F	45	740	Myocardial hypertrophy and dilatation, generalized arteriosclerosis
16	M	69	550	Myocardial hypertrophy and dilatation, generalized arteriosclerosis
17	M	55	800	Myocardial hypertrophy and dilatation, generalized arteriosclerosis

calculation one millimol of phosphorus is considered one milliequivalent (see Peters and Van Slyke (1931), p 1102) The average, maximum and minimum values for the several elements are summarized in Table III In addition, in this table, is given a similar summary based on dry weight because much of the previous work has been reported in dry weight

Water

Domagk (1924) and Scott (1931-a) have shown that the water content of the left ventricles of persons who died of tuberculosis was greater than that of the left ventricles of persons dying from a variety of other diseases Apparently neither of these workers determined the water content of the right ventricles Scott (1930, 1931-a) found no difference in the average water content of the left ventricles of cardiac heart and that from a number of persons who died from other diseases

TABLE II
Inorganic constituents of ventricles of normal and diseased hearts

	Right ventricle						Left ventricle					
Per 100 grams wet tissue												
Case number	H ₂ O grams	P mgm	K mgm	Na mgm	Ca mgm	Mg mgm	H ₂ O grams	P mgm	K mgm	Na mgm	Ca mgm	Mg mgm
Normals												
1	80.1	203	229	96	8.2	21.8	78.1	242	324	73	6.6	23.2
2	80.4	164	259	133	9.3	18.2	79.5	184	280	114	9.5	18.8
3	76.9	180	285	111	7.5	20.3	78.0	199	325	98	6.3	20.4
4	81.2	142	220	112	7.0	16.4	80.1	179	302	97	6.2	15.6
5	77.6	195	280	83	5.9	22.3	78.8	213	326	76	6.0	22.6
Average	79.2	177	255	107	7.6	19.8	78.9	203	311	92	6.9	20.1
Intermediate												
6	80.9	137	205	148	8.2	15.0	79.8	177	262	118	6.3	18.6
7	83.7	159	201	181	6.2	14.6	80.9	213	332	106	6.4	20.2
8	83.0	146	178	130	9.2	15.7	80.3	154	217	81	6.4	16.5
9	79.0	146	203	138	9.4	15.8	78.9	197	326	101	6.9	19.4
Average	81.7	147	197	149	8.3	15.3	80.0	185	284	102	6.5	18.7
Cardiacs												
10	82.0	143	223	129	6.4	15.1	82.1	149	237	128	5.6	15.4
11	82.3	152	209	155	5.4	13.8	80.4	178	226	115	4.4	15.7
12	83.6	122	134	186	8.9	11.6	81.9	142	209	154	6.7	14.0
13	82.9	150	174	153	9.9	14.6	81.5	176	236	132	10.9	15.6
14	82.4	142	180	147	7.2		80.4	173	277	92	5.6	
15	81.4	145	181	148	5.5		79.1	170	248	102	5.0	
16	78.2	158	235	120	8.7	14.6	78.9	185	325	94	7.1	18.0
17	79.3	183	265	101	4.5	18.5	79.4	188	303	100	4.4	16.3
Average	81.5	149	200	142	7.1	14.7	80.5	170	258	115	6.2	15.8

In the present study there is no evidence from the 5 normal hearts of a consistent variation in water content between right and left ventricles, but for the diseased group there is a definite tendency for each right ventricle to have a higher water content, by about 1 per cent, than that of the left from the same heart. Moreover, the heart from persons dying with congestive heart failure showed an average of about 2.3 per cent more water in the right ventricles and about 1.6 per cent more in the left ventricles than did the normal hearts. These changes stand out more emphatically when stated in terms of percentage solids. This

TABLE III

Averages of inorganic constituents of ventricles calculated on basis of dry-weight and of tissue water

Group	Mgm per 100 grams dry tissue		m Eq per kilo tissue H O	
	Right ventricle	Left ventricle	Right ventricle	Left ventricle
<i>Phosphorus</i>				
Normal average	852	962	72	83
Intermediate average	811	926	58	74
Cardiac average	808	870	59	68
<i>Potassium</i>				
Normal average	1225	1475	83	100
Intermediate average	1081	1422	61	91
Cardiac average	1075	1314	63	82
<i>Sodium</i>				
Normal average	521	436	58	51
Intermediate average	828	508	79	55
Cardiac average	786	594	76	62
<i>Calcium</i>				
Normal average	36.9	32.7	4.8	4.4
Intermediate average	45.0	29.9	5.1	4.1
Cardiac average	38.8	33.4	4.3	3.9
<i>Magnesium</i>				
Normal average	95.4	95.0	20.6	21.0
Intermediate average	84.0	93.5	15.4	19.2
Cardiac average	79.0	82.0	14.9	16.2

means a decrease from normal of 11 per cent of the total solids in the right ventricles and of 7.5 per cent in the left ventricles. Apparently the right ventricle shows a greater susceptibility to increase in water content or decrease in solids than does the left ventricle.

This is in agreement with the findings of Calhoun, Cullen, Clarke and Harrison (1930) who found that right and left ventricles in patients who died with congestive heart failure showed an increase in water content over the hearts of patients who died from a variety of other diseases. It is also of interest to note that the two right ventricles which contained the most water were also high in sodium. Of these, one showed also the lowest potassium content on any basis of calculation. This right

ventricle (case number 12), was also the lowest in magnesium and phosphorus per unit of wet weight. These facts indicate a definite tendency toward edema in diseased heart muscle, especially that of the right ventricle.

Phosphorus

The figures for phosphorus represent total phosphorus in each case, no attempt was made to determine the phosphorus fractions.

Scott (1931 *a*) reported a large series of phosphorus determinations on the left ventricles of persons who died with a variety of diseases and a group who died of tuberculosis. The following is a summary of his values.

	Maximum	Minimum	Mean
	<i>mgm per 100 grams of fresh tissue</i>		
General diseases	233	139	176
Tuberculosis	193	129	153

In the present normal group the left ventricles contained an average of about 15 per cent more phosphorus than did the right. In the group of persons who died with congestive heart failure the left ventricles contained an average of about 14 per cent more phosphorus than the right. Both the right and left ventricles of the persons who died with congestive heart failure contained an average of about 16 per cent less phosphorus than did the corresponding ventricles of the normal group. These findings are in agreement with those of Laszlo (1928) who noted a decreased amount of phosphorus in the skeletal and cardiac muscles of persons who died with cardiac disease.

Potassium

In this study the individual left ventricles of each group showed without exception a higher concentration of potassium than did the right. This contrasts with the fact that the left ventricles of each group without exception showed a lower content of sodium than did the right. One case with extreme congestion of the lungs showed very low values for potassium and high values for sodium in both ventricles, but especially in the right.

When these values are expressed in milliequivalents per kilo of muscle water, the total concentration of sodium plus potassium in the right ventricles is of the same order as that of the left ventricles.

The hearts of individuals who died with congestive heart failure were poorer in potassium and richer in sodium than those of the normal control group. The figures show that there was some degree of overlapping, but the averages show a definite and appreciable difference.

This is true when the concentrations are expressed in milligrams per 100 grams of fresh tissue, milligrams per 100 grams of dried tissue, and in miliequivalents per kilo of tissue water

These findings, in respect to potassium, confirm, with a different analytical method, those of Harrison, Pilcher and Ewing (1930), and those of Calhoun, Cullen, Clarke and Harrison (1930) The latter authors believe that overwork causes loss of potassium from cardiac muscle and that this loss is one of the predisposing factors to cardiac fatigue and failure They also studied a number of different tissues from individuals who died of a variety of diseases, and suggest that potassium loss in congestive heart failure is not confined to the heart and skeletal muscle, but apparently holds for several other types of tissue Later Calhoun, Cullen and Harrison (1930) found that overwork of the muscles of one leg of dogs, produced by continual stimulation of the sciatic nerve causing the leg to lift a weight, usually leads to a diminished content of potassium in the muscles as compared with those of the opposite unstimulated leg

Harrison, Pilcher, and Ewing (1930) suggested the possibility that loss of potassium from the heart muscle is compensated for by an increase in one or more of the other basic elements The present study shows that there is a "compensation" by sodium

Scott (1931-b) who has made the most extensive study of the inorganic substances in human left ventricle heart muscle, failed to find a significant diminution of potassium in the left ventricles of persons who died of congestive heart failure compared with the left ventricles of persons who died from a variety of other diseases He found highly variable amounts of both sodium and potassium in both groups His average values for potassium in the left ventricle are much lower than those of other authors, including ourselves

The discrepancy between Scott's values (1931-b) for potassium and the present results has been disturbing The possibility that this is due to a difference in ashing is suggested by the following observations When the present investigation was begun, a number of tissues were ashed in somewhat the same manner as were those of Scott, and results for potassium similar to his were obtained These results were low as compared with the previous studies in this laboratory, and the ashing technic was scrutinized It was found that if ashing were done in the muffle furnace without H_2SO_4 , the potassium values were low When a small amount of H_2SO_4 was added before ashing the duplicate ashings gave potassium figures which were higher, which checked closely, and which were within the range of those previously obtained when the wet ashing technic was used Blanks were, of course, run under both sets of conditions

In a more recent study with an improved method Scott (1931-c) reported much higher values for potassium in the left ventricles of a large number of hearts than he reported in the three previous studies These later values are more nearly within the range of ours

Sodium

Few figures are available on the sodium content of the various soft tissues, although its concentration in the blood has been the object of numerous investigations.

In the human, sodium appears to be present in much greater concentrations in the extracellular fluids than in any of the cells. In this respect it differs from potassium and magnesium, which appear in greater concentrations in the cellular structures. Of the anions, chloride appears to vary with sodium, while phosphate varies with potassium.

Loeb, Atchley and Palmer (1922) have shown that edema fluids may contain approximately the same concentration of sodium as blood serum. It follows that even a slight increase in the water content of a tissue may be accompanied by an appreciable increase in sodium.

In the present study the sodium content of the right and left ventricles of seventeen human hearts was determined. In all seventeen cases, with two exceptions, the right ventricles contained decidedly more sodium than did the left ventricles. In every instance, the opposite was true of potassium.

In both ventricles the normal hearts showed a higher average content of potassium than did the corresponding ventricles of persons who died with congestive heart failure. Just the reverse was the case with sodium. There was some overlapping with reference to sodium as well as with potassium, but most of the individual cases, as well as the averages, showed a definite increase in the sodium content of both ventricles of the persons who died with congestive heart failure.

The four left ventricles which had the highest potassium content showed the lowest values for sodium.

Scott (1930) reported a number of instances in which the normal K/Na ratios were almost reversed in the left ventricle. We found some tendency toward such a reversal in both ventricles of several persons who died with congestive heart failure, but found no such change in any heart in the normal group.

Scott's averages for the sodium content of the left ventricles are higher than those yielded by the present study. In view of the fact that his values for sodium were calculated by difference, his higher figures for this element may have been due to his failure to recover all of the potassium. This point has been discussed in the section on potassium.

Calcium and magnesium

Although the significance of changes in calcium concentration in the blood has been studied intensively, little is known concerning changes of calcium in tissue. Little is known concerning the importance of magnesium in either blood or tissue. Magnesium is essentially a tissue cation, calcium is in greater concentration in serum and other extracellular

fluids than it is in most soft tissues. Katz showed that human skeletal muscle contained about 7.5 mgm per cent of calcium and 21 mgm of magnesium per 100 grams fresh muscle. On a basis of milliequivalents this gives a Ca/Mg ratio of about 1-4. Scott found in the left ventricles of 14 hearts from patients with general disease an average of 8 mgm of calcium and 17.4 mgm of magnesium per 100 grams fresh tissue and in 14 hearts from patients dying with tuberculosis 9.0 mgm calcium and 16 mgm magnesium.

In another study (Cullen, Wilkins and Harrison (1933)) we found in autopsies from general disease that the right ventricles average 7.3 mgm per cent of calcium and 18.4 mgm per cent magnesium, and the left ventricles averaged 6.4 mgm per cent calcium and 21 mgm per cent magnesium. In the present study 15 of the 17 hearts show slightly less calcium and more magnesium in the left ventricle than in the right.

The variation in calcium content in both normal and diseased hearts is so great that, with the small number of cases, it is impossible to state whether or not there is less calcium in the diseased heart than in the normal. There is a tendency for the abnormal hearts to show a decrease in calcium proportional to the decrease in total solids which suggests that the calcium is related to the tissue protein in the same manner that it is related to serum protein. Scott, however, found no significant difference between the calcium content of the left ventricles of patients dying of heart failure and of those dying from other diseases.

In most cases the hearts showing cardiac disease have a decidedly lower magnesium content than do the normal hearts.

In general the variations of magnesium content parallel those of potassium and phosphorus.

GENERAL DISCUSSION

Total base determinations, as such, were not made on this group of tissues, but were calculated from the values for the individual bases. We found, in a former study, that some calcium and magnesium failed to come through during the removal of phosphate by the method of Stadie and Ross (1925). This fact has also been noted by Brown and Shohl (1931).

In all of the normal group the concentration of total bases (in milliequivalents per kilo of tissue water) was higher in the left ventricle than in the right, but both were within the same general range.

The right ventricles of the normal hearts show an average of 167 and the left ventricles an average of 176 milliequivalents per kilo of tissue water. The hearts from persons who died with congestive heart failure contained an average of 159 milliequivalents per kilo of tissue water in the right ventricles and 166 in the left. Thus the latter group showed in both ventricles a slight diminution in total base as compared with the normal controls.

Van Slyke Wu and McLean (1923) have pointed out that the osmolar concentration of total electrolytes in the water of blood plasma and cells is practically equal despite the differences in the concentrations of the individual ions. They found, in a typical case, 162 milliequivalents of base per kilo of serum water and 174 per kilo of red blood cell water. Gamble, Ross and Tisdall (1923) have expressed the belief that this close relationship holds also for the cell water of muscles and probably for other tissues of the body. The present results support this view.

The total electrolyte concentration in the tissue is of importance in relation to the osmotic conditions existing there. Although the data given above do not give any direct evidence as to the cause of the shift in diseased tissue, it is significant that it is precisely what would be expected if the permeability of the tissue were increased. Then sodium would diffuse into the tissue cells from its higher concentration in extracellular fluids, and potassium, magnesium and phosphates would diffuse out from their higher concentration in the cells. Calcium, approximately equal in all fluids, would show little change. The relations between potassium, phosphorus and sodium are shown graphically in Figure 1. It is at once apparent that potassium and phosphorus parallel each other and that sodium fluctuates regularly in the opposite direction to potassium and phosphorus.

The question of the ratio of the individual elements to each other is of course important and the average ratios of P/K and K/Na are given in Table IV.

TABLE IV

Ratio of potassium to phosphorus and sodium based on concentration per kilo tissue water

	$\frac{\text{Millimols P}}{\text{Millimols K}}$		$\frac{\text{Milliequivalents K}}{\text{Milliequivalents Na}}$	
	Right ventricle	Left ventricle	Right ventricle	Left ventricle
Normal average	0.88	0.83	1.45	2.05
Intermediate average	0.96	0.82	0.78	1.65
Cardiac average	0.96	0.84	0.88	1.39

The data demonstrates unmistakably a real difference in the concentration of the important electrolytes in the two ventricles. It is important that both potassium and phosphorus, which are known to be involved in the buffer mechanism of muscle are in the muscle which has the greatest work load. However, until the phosphorus distribution in these conditions is known, it is undesirable to speculate further.

We wish to acknowledge our indebtedness to Dr. Richard Austin of the University of Cincinnati, Drs. George M. Leiby and Kenneth M. Lynch of the Medical College of the State of South Carolina. Dr. C. W.

Averages for P, K, and Na

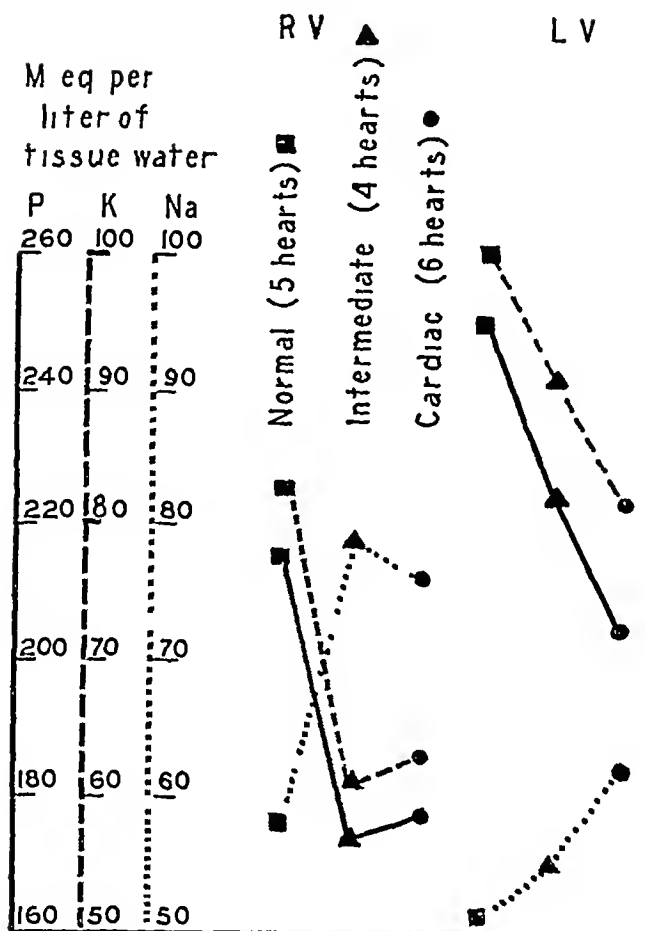


FIG 1

Muehlberger of the Coroner's Laboratory, Cook County Morgue, Chicago, and to the Department of Pathology of the Vanderbilt School of Medicine, for furnishing us the tissues used in this study

SUMMARY

1 The right and left ventricles of seventeen human hearts were analyzed for water, phosphorus, sodium, potassium, magnesium and calcium. Of these hearts, five were normal, four were from persons who had cardiac disease but who died from other causes, and eight were from persons who died with congestive heart failure.

2 The water content of the diseased right ventricle tends to be slightly higher than that of the left ventricle. The water content of both ventricles of hearts from persons who died with congestive heart failure was found to be increased.

3 The normal left ventricle contains more total phosphorus and more potassium than the right. Both ventricles of diseased hearts showed a decrease in total phosphorus and potassium.

4 The normal right ventricle contains more sodium than the normal left ventricle. Sodium was increased in both ventricles of persons who died with congestive heart failure.

5 Usually the right ventricle contains a slightly higher concentration of calcium than does the left ventricle. No consistent variations were found in the calcium content of the ventricles of individuals who died with congestive heart failure.

6 Both the normal and diseased left ventricles were richer in magnesium than the corresponding right ventricles. Both ventricles of the diseased hearts showed a diminution in magnesium.

7 The sums of the individual bases when calculated in milliequivalents per kilo of tissue water show that the two ventricles do not differ essentially in their content of total base.

8 The K/Na ratio in the normal left ventricle is higher than in the normal right ventricle. Both ventricles of the diseased hearts showed a decrease in the K/Na ratio.

9 The P/K ratio was somewhat higher in the right ventricle than in the left. The diseased hearts had nearly the same P/K ratios in both ventricles as did the normal hearts, showing that the former had a proportionate decrease in phosphorus and potassium.

BIBLIOGRAPHY

- Brown, H. B., and Shohl, A. T., *J. Biol. Chem.*, 1931, **xc**, 745. The Determination of Sodium Plus Potassium as Benzidine Sulfate.
- Calhoun, J. A., Cullen, G. E., Clarke, G., and Harrison, T. R., *J. Clin. Invest.*, 1930, **ix**, 393. Studies in Congestive Heart Failure. VI. The Effect of Overwork and Other Factors on the Potassium Content of the Cardiac Muscle.
- Calhoun, J. A., Cullen, G. E., and Harrison, T. R., *J. Clin. Invest.*, 1930, **ix**, 405. Studies in Congestive Heart Failure. VII. The Effect of Overwork on the Potassium Content of Skeletal Muscles.
- Cullen, G. E., Wilkins, W. E., *J. Biol. Chem.*, 1933, **ci**, 403. Electrolytes in Human Tissue. I. The Digestion of Tissue and Other Biological Material and the Subsequent Determination of Various Electrolytes.
- Cullen, G. E., Wilkins, W. E., and Harrison, T. R., *J. Biol. Chem.*, 1933, **ci**, 415. Electrolytes in Human Tissue. II. The Electrolyte Content of Hearts and Other Tissues from Various Diseases.
- Domagk, G., *Ztschr. f. klin. Med.*, 1924, **xcviii**, 171. Die chemische Zusammensetzung des Herzmuskels bei verschiedenen Erkrankungen.
- Gamble, J. L., Ross, G. S., and Tisdall, F. F., *J. Biol. Chem.*, 1923, **lvii**, 633. The Metabolism of Fixed Base During Fasting.
- Harrison, T. R., Pilcher, C., and Ewing, G., *J. Clin. Invest.*, 1930, **viii**, 325. Studies in Congestive Heart Failure. IV. The Potassium Content of Skeletal and Cardiac Muscle.
- Katz, J., *Arch. f. d. ges. Physiol.*, 1896, **lxiii**, 1. Die mineralischen Bestandtheile des Muskelfleisches.

- Laszlo, D , Klin Wchnschr , 1928, vii, 1411 Stoffwechseluntersuchungen bei Herzkranken
- Loeb, R F , Atchley, D W , and Palmer, W W , J Gen Physiol , 1922, iv, 591 On the Equilibrium Condition Between Blood Serum and Serous Cavity Fluids
- Peters, J P , and Van Slyke, D D , Quantitative Clinical Chemistry, I Interpretations Williams and Wilkins Co , Baltimore, 1931
- Scott, L C , J Clin Invest , 1930, ix, 463 The Proportion of Certain Inorganic Constituents in the Dying Heart Muscle
- Scott, L C , (a) Am Rev Tuberc , 1931, xxi, 429 The Proportions of Water and Other Inorganic Constituents in the Heart Muscle of Tuberculous Individuals
- Scott, L C , (b) Arch Int Med , 1931, xlii, 116 The Potassium Content of the Hearts of Persons Dying from Edematous and Non-edematous Conditions
- Scott, L C , (c) J Clin Invest , 1931, x, 745 The Determination of Potassium in Cardiac Muscle and the Presumable Influence of the Beta Radiations on the Rhythm
- Stadie, W C , and Ross, E C , J Biol Chem , 1925, lxxv, 735 A Micro Method for the Determination of Base in Blood and Serum and Other Biological Materials
- Van Slyke, D D , Wu, H , and McLean, F C , J Biol Chem , 1923, lvi, 765 Studies of Gas and Electrolyte Equilibria in the Blood V Factors Controlling the Electrolyte and Water Distribution in the Blood

THE CISTERNAL PRESSURE IN CONGESTIVE HEART FAILURE AND ITS BEARING ON ORTHOPNEA

By WILLIAM GROCE HARRISON, JR

(From the Department of Internal Medicine, Emory University School of Medicine Grady Hospital (colored unit), Atlanta)

(Received for publication July 12 1933)

Various theories concerning the cause of orthopnea have been advanced, but sufficient proof for accepting any one explanation of this phenomenon has not been offered

As an excellent review of the literature on orthopnea has been given by Ernstene and Blumgart (1), only a few of the theories will be mentioned here Sahli (2) thought that the greater use of the accessory muscles of respiration, the accumulation of blood in the lower extremities leading to diminished pulmonary congestion, and a decrease in the work of the heart along with the relief of venous congestion of the brain and of the respiratory center by the action of gravity were the fundamental factors responsible for the sitting posture in orthopnea Haldane, Meakins and Priestly (3) expressed the belief that a decrease in anoxemia of the respiratory center and a better oxygenation of the arterial blood leaving the lungs were largely responsible for the greater comfort in breathing that a patient with cardiac disease exhibits in the upright position

Christie and Beams (4) reported careful studies of the vital capacity of a large number of normal subjects and of patients with cardiac disease They found that, although the vital capacity was decreased when the normal person went from sitting to prone position, this decrease was much greater in patients with cardiac disease accompanied by orthopnea They, therefore, inferred that the diminished vital capacity of the lungs in the prone position is the main cause of orthopnea

Ernstene and Blumgart (1), however, observed *orthopnea of necessity* in patients in whom the vital capacity of the lungs was not significantly increased by changing from the recumbent to the sitting position They demonstrated furthermore a parallelism between the height of venous pressure and the degree of orthopnea in twenty two patients with uncomplicated myocardial failure They were inclined to the view that lowering of cerebral venous pressure in the sitting posture is the primary factor in reducing the respiratory discomfort

In a recent series of studies Harrison (5) demonstrated an increase in cerebrospinal fluid pressure in patients with cardiac failure and the parallelism of venous and spinal fluid pressure in this condition He

also observed the effect of treating by means of spinal drainage patients having congestive heart failure. It was noted in observations not yet published that after spinal drainage was performed on individuals with orthopnea many of these patients were able to breathe more comfortably in the recumbent position, although the vital capacity of the lungs had not been significantly altered by this procedure. A study was also made of the cisternal pressure in the upright and recumbent positions in one normal individual, and in one patient with congestive heart failure, and the relation of cisternal pressure to orthopnea was discussed. In the present paper a larger group of such studies is reported.

The cisternal pressure in patients without cardiac disease

While performing routine lumbar punctures on patients suspected of having syphilis an opportunity was offered to measure the cisternal pressure and the spinal fluid pressure in the sitting and prone positions in five patients, none of whom had any evidence of cardiac disease. A U-shaped glass manometer filled with normal saline was used to make the readings of cisternal pressure. The readings of lumbar spinal fluid pressure were taken with an Ayer water manometer. Venous pressures were determined by the direct method of Moritz and Tabora observing the usual precautions. The vital capacity, the arterial blood pressure, and the pulse and respiration rates were likewise recorded in both positions (Table 1).

The systemic venous pressure, as measured at the elbow, was found to be slightly higher in the upright than in the recumbent position. The lumbar spinal fluid pressure was much greater in the sitting position. The cisternal pressure (which ranged from -22 to $+17$) was on the other hand much lower in the sitting position, and in one individual was below zero. The average arterial blood pressure and pulse rate were slightly greater in the sitting position, but neither the blood pressure nor pulse or respiration rates showed marked consistent changes, on the other hand in all instances the vital capacity was slightly increased in the sitting position.

The cisternal pressure in patients with congestive heart failure

An opportunity was offered to carry out similar observations on five patients who had either syphilitic aortic insufficiency or hypertensive heart disease with congestive failure, while attempts were being made to give relief by spinal drainage. Similar measurements were made in the manner described above. All observations on any one patient were performed on the same day¹ (Table 2).

¹ The cisternal pressure recorded was slightly lower in some instances and the spinal fluid pressure lower in others than they should have been because in some cases the spinal fluid pressure was taken first while in others the cisternal pressure was measured first.

TABLE 1
Control group

	Venous pressure		Spinal fluid pressure		Cisternal pressure †		Vital capacity		Arterial blood pressure		Pulse		Respirations	
	P*	S*	P	S	P	S	P	S	P	S	P	S	P	S
J R Syphilis	65	100	60	350	125	10	2800	2900	108/90	130/100	72	76	24	20
T H Catarrhal jaundice	50	70	125	430	102	15	1900	2000	100/64	110/70	80	92	20	24
W F Duodenal ulcer	45	80	50	420	65	-22	3300	3450	108/80	106/80	88	100	16	20
F O Duodenal ulcer	55	70	65	380	65	17	2200	2300	108/76	108/85	102	100	20	20
S S Arspenamine hepatitis	50	50	140	450	110	15	3450	3550	104/60	106/76	70	74	12	16
Average	52	74	88	406	93	7	2730	2840	105/74	112/82	82	88	18	20

* P—prone

S—sitting

† Note the difference in the cisternal pressure in the two positions in each patient

TABLE 2

Congestive heart failure group

	Venous pressure		Spinal fluid pressure		Cisternal pressure †		Vital capacity		Arterial blood pressure		Pulse		Respirations	
	P*	S*	P	S	P	S	P	S	P	S	P	S	P	S
	mm H ₂ O	mm H ₂ O	mm H ₂ O	mm H ₂ O	mm H ₂ O	mm H ₂ O	cc	cc	mm Hg	mm Hg				
DH Syphilitic aortic insufficiency	215	230	320	415	310	50	900	1200	134/52	144/58	88	100	28	40
TW Hypertensive heart disease	310	355	400	680	355	100	1200	1400	158/128	152/116	60	86	40	44
HL Hypertensive heart disease	200	260	320	640	295	65	1000	1100	190/94	188/90	100	110	28	34
FW Hypertensive heart disease	310	345	360	480	365	80	1700	1900	160/100	154/98	80	88	20	22
LP Syphilitic aortic insufficiency	335	375	450	660	360	110	1100	1500	220/64	220/64	100	100	28	26
Average	274	313	374	575	337	81	1180	1420	172/87	171/85	85	96	28	33

* P—prone

S—sitting

† Note the marked difference in the cisternal pressure as well as the vital capacity in the two positions in each patient. This difference in the cisternal pressure in the two positions is much greater than in the control group.

The venous pressure, the lumbar spinal fluid pressure, and the cisternal pressure in each position were found to be much greater in the patients with cardiac disease than in the normal individuals. The venous and the lumbar spinal fluid pressures in the patients with cardiac disease were also considerably higher in the sitting than in the prone position. As in the first group the cisternal pressure was much less in the upright than in the recumbent position. Although the difference in the cisternal pressure in the two positions in the normal individuals was considerable, in the patients with cardiac disease this difference was much greater and averaged 256 mm water. The arterial blood pressure, and pulse and respiration rates as in the first group showed no marked consistent changes between the two positions, although the pulse rate was usually slightly greater in the sitting position. In every case the vital capacity was higher in the sitting than in the prone position and the percentile increase in the vital capacity was greater in this group than in the patients without cardiac disease.

COMMENT

Observations on the cisternal pressure of the normal individual in the sitting position have been rare. Ayer (6) reported the cisternal pressure in the upright position to be below zero. Weed (7) demonstrated the difference in cisternal pressure in animals in the two positions, and Weed and Hughson (8) have shown the close relationship that exists between the cerebrospinal fluid pressure and cerebral venous pressure. It has been shown by Harrison, Cullen, Calhoun, Wilkins and Pilcher (9) that a decrease in vital capacity tends to cause a reflex increase in breathing. That an increase in venous pressure causes a reflex stimulation of respiration has been demonstrated by Harrison, Harrison, Calhoun, and Marsh (10).

Since the vital capacity is less in the prone than in the sitting position in patients with congestive heart failure, this decrease in vital capacity is no doubt one important cause of orthopnea. Furthermore, patients have been observed with slight orthopnea who have a diminution in vital capacity but no significant elevation in venous pressure or in cerebrospinal fluid pressure.

On the other hand, a number of observations point to the conclusion that there is another very important factor not connected with the vital capacity concerned in the production of orthopnea. (1) In the first place, Ernstene and Blumgart observed no parallelism between the percentile reduction of vital capacity and the degree of orthopnea, but did observe a striking parallelism between the height of venous pressure and the degree of orthopnea. (2) Ernstene and Blumgart found that when patients with orthopnea were placed in the recumbent position, simple elevation of the head by flexing it on the thorax produced a

conspicuous diminution of respiratory distress, although this procedure had no significant effect on the vital capacity of the lungs (3) Harrison found that, after spinal drainage was performed on individuals with orthopnea, many of these patients were able to breathe more comfortably in the prone position, although the vital capacity showed no significant change after this procedure. It has now been shown that the cisternal pressure is markedly decreased when a patient goes from the prone to the sitting position. It seems probable that this decrease in cisternal pressure along with its accompanying change in cerebral venous pressure explains the three sets of observations just mentioned. Anything that tends to decrease cisternal pressure in a patient with cardiac disease will give additional comfort in breathing. One method of reducing this pressure is to sit the patient up. Another method is to employ spinal drainage. Obviously, venesection is very useful, since it not only decreases venous and spinal fluid pressures, but also increases vital capacity.

As a result of these observations it is believed that, in addition to the increase in vital capacity, the diminished cisternal pressure is an important factor in producing the relief from dyspnea obtained in the sitting position. The mechanism of this effect is as yet unknown.

SUMMARY AND CONCLUSIONS

A study was made of the cisternal pressure, the lumbar spinal fluid pressure, the systemic venous pressure, the vital capacity, the arterial blood pressure, and the pulse and respiration rates in the recumbent and upright positions in five patients without cardiac disease and in five patients with congestive heart failure.

1 The arterial blood pressure and pulse rate were usually slightly greater in the sitting than in the prone position, but in general the blood pressure, and the pulse and respiration rates showed no marked consistent changes with position in either group.

2 The systemic venous and lumbar spinal fluid pressures were greater in the sitting than in the prone position in each subject, and were much greater in each position in the patients with congestive heart failure than in the patients without cardiac disease.

3 The vital capacity was greater in the sitting than in the prone position in both groups of cases and the percentile increase of vital capacity with change in position was greater in the patients with cardiac disease.

4 The cisternal pressure was much greater in each position in the group with congestive heart failure than in the other group.

5 The cisternal pressure was much less in the upright than in the recumbent posture in each case and this difference in cisternal pressure between the two positions was much greater in patients with congestive heart failure than in those without cardiac disease.

As a result of these observations it is believed that, in addition to the increase in vital capacity, the diminished cisternal pressure is an important factor in producing the relief from dyspnea obtained in the sitting position. The mechanism of this effect is as yet unknown.

BIBLIOGRAPHY

- 1 Ernstene, A C, and Blumgart, H L, *Arch Int Med*, 1930, xlv, 593
Orthopnea—Its Relation to the Increased Venous Pressure of Myocardial Failure
- 2 Sahli, H, *Lehrbuch der klinischen untersuchungs Methoden* Franz Deuticke, Leipzig, 1894
- 3 Haldane, J S Meakins, J C, and Priestley, J G, *J Physiol*, 1919, lii 433 The Effects of Shallow Breathing
- 4 Christie, C D, and Beams, A J, *Arch Int Med*, 1923, xxi, 85
Orthopnea
- 5 Harrison, W G, Jr, *Arch Int Med*, (In press) Cerebrospinal Fluid Pressure and Venous Pressure in Cardiac Failure and the Effect of Spinal Drainage in the Treatment of Cardiac Decompensation
- 6 Ayer, J B, *The Human Cerebrospinal Fluid* 1926, Chap xi, p 159
- 7 Weed, L H, *Physiol Rev* 1933, xii, 80 Positional Adjustments of the Pressure of the Cerebrospinal Fluid
- 8 Weed, L H, and Hughson, W *Am J Physiol*, 1921-22, lvi, 101
Intracranial Venous Pressure and Cerebrospinal Pressure as Affected by the Intravenous Injection of Solutions of Various Concentrations
- 9 Harrison, T R, Calhoun, J A, Cullen G E Wilkins, W E, and Pilcher, C, *J Clin Invest.*, 1932, xi, 133 Studies in Congestive Heart Failure XV Reflex Versus Chemical Factors in the Production of Rapid Breathing
- 10 Harrison, T R, Harrison, W G, Jr, Calhoun, J A, and Marsh, J P, *Arch Int Med*, 1932, l, 690 Congestive Heart Failure XVII The Mechanism of Dyspnea on Exertion

THE ACTION OF PHLORIZIN ON THE EXCRETION OF GLUCOSE, XYLOSE, SUCROSE, CREATININE AND UREA BY MAN

By HERBERT CHASIS NORMAN JOLLIFFE AND HOMER W SMITH

(From the Department of Physiology New York University and Bellevue Hospital Medical
College, New York City)

(Received for publication July 21, 1933)

Although phlorizin has been used mostly in the lower animals, its administration to man is not without precedent, shortly after its isolation by de Koninck (1836a, b), this investigator tried it in the treatment of malaria on the ground that it was bitter, like other remedies which were effective in this disease. The use of the drug in this connection was short-lived, however, and it was not until many years later that v Mering (1885) discovered that it caused glycosuria and diuresis, observations which initiated its application in the treatment of nephritis, sarcoma, etc., and as a test for renal function in man, in addition to its well known use in studies of metabolism. Since these instances of human administration are of some interest, we have summarized them in Table I.

The largest dose *per os* appears to be 15 grams in wafer form, reported by Pietkiewicz (1869) and v Mering (1889), while Korte (1896) took three five gram doses dissolved in alcohol in one day. v Mering (1889) also records the largest parenteral dose (in a case of sarcoma) 2 grams (in warm aqueous solution) injected subcutaneously daily, one gram in the morning and one gram in the evening, for a period of thirty days. Benedict and Lewis (1913—personal communication) gave 2 grams daily in 10 cc of sterile olive oil subcutaneously over a period of days. In no instance was permanent injury described.

Moderate intravenous doses of the drug produce no unfavorable reactions in the dog, and we decided that the intravenous route would be the most satisfactory for our purposes, particularly since we wished to determine the exact effect of small doses. Our anticipation in this matter has been justified, since we have given phlorizin in doses varying from 20 to 650 mgm per kgm with no unfavorable reactions other than an apparent diminution in glomerular activity with the larger doses. We feel called upon, however, to express a word of caution about the use of the intravenous technique in man and refer the reader to the description of our method of preparation and administration. Previous observations from this laboratory have shown that in the dog adequate doses of phlorizin raise the glucose clearance and lower the creatinine clearance to the level of the xylose clearance (Jolliffe, Shannon and Smith

TABLE I
Administration of phlorizin to man

Investigator	Date	Mgm per man per diem	Route of administration
de Koninck	1836	1,600	Per os, divided doses
v Coetsem	1836	1,600	Per os, one dose
Mareska	1836	1,600	Per os, 2 doses
de Muynck	1836	1,250	Per os, ?
Hanegraeff	1836	1,600	Per os, one dose
Pietkiewicz	1869	15,000	Per os, one dose
v Mering	1889	15,000	Per os, one dose
Klemperer	1896	10,000	Per os, one dose
Korte	1896	15,000	Per os, 3 doses
v Mering	1889	2,000	Hypodermic, 2 doses, for 30 days
Magnus-Levy	1896	1,000	Hypodermic, ?
Achard and Delamare	1899	50	Hypodermic, one dose
Delamare	1899	50	Hypodermic, one dose
Casper and Richter	1900	?	Hypodermic, one dose
Allard	1907	200	Hypodermic, one dose
Tanaka	1908	10	Hypodermic, one dose
Benedict and Lewis	1913	2,000	Hypodermic, one dose, repeatedly*
Chabanier and Lobo-Onell	1913	25	Hypodermic, one dose
Grote	1921	10	Hypodermic, one dose
Kamnitzer and Joseph	1921	25	Hypodermic, one dose
Hetényi	1922	60	Hypodermic, one dose
Rosenberg	1923	50	Intramuscular, one dose
Kamnitzer and Joseph	1924	2	Intramuscular, one dose
Dunner and Mecklenburg	1925	44	Hypodermic, one dose
Dunner and Kronenberger	1930	10	Intramuscular, one dose
Present experiments	1933	3,800	Intravenous, one dose

* In oil (personal communication)

(1932) and Shannon, Jolliffe and Smith (1932)) Our present experiments were designed to determine if similar changes in the glucose and creatinine clearances occur in man. Large doses of the drug were avoided, and different individuals were employed for each test.

METHODS

Our subjects were convalescent patients, with normal renal function, kept in bed throughout the morning of the experiment. The routine of the experiments listed in Table II was as follows:

The patient was allowed no breakfast. At zero minutes 70 grams of xylose were given in 10 tablespoons of oatmeal (no milk or sugar) and shortly afterward 10 grams of creatinine were taken in water. (N.B. The administration of xylose in oatmeal circumvents the diarrhea which frequently follows its administration in aqueous solution and generally gives a flat blood sugar curve.) Varying amounts of water were given at zero, 30 and 60 minutes, and at 90 minutes the bladder was emptied (*vide infra*) and the first urine collection period begun. After one or more control periods phlorizin was injected

intravenously During the next few minutes (washout period) normal saline was instilled into the bladder and removed with the discard Three more specimens of urine were then collected at intervals of 20 to 30 minutes each

In the experiments listed in Table III the above routine was modified to permit the intravenous injection of sucrose At zero minutes 500 cc of water were taken followed by 70 grams of xylose in 10 tablespoons of oatmeal, at 50 minutes an intravenous infusion of 10 per cent sterile sucrose solution (in distilled water) was begun, 600 cc being injected over a period of 30 to 50 minutes Immediately after this injection, phlorizin was administered intravenously

Urine was collected by catheterization in dry flasks containing a little benzoic acid with special care to secure complete emptying of the bladder Samples of blood were taken from the antecubital vein as near to the middle of each urine collection period as possible The blood was centrifuged and the plasma precipitated at once All plasma concentrations were interpolated to the exact middle of the urine collection period and no correction was made for urinary dead space

Phlorizin, repurified by the method of Deuel and Chambers (1925) was weighed out in advance of the experiment Just before use the phlorizin was dissolved in hot, freshly boiled 2.5 per cent NaHCO_3 , and while still warm the solution was injected into the antecubital vein In those experiments where the dose of phlorizin was greater than 2.5 grams, it was necessary to weigh the drug in two portions and to dissolve them separately (Otherwise the solution cools and the drug recrystallizes during the injection)

The chemical methods used were those of Jolliffe Shannon and Smith (1932) and Shannon, Jolliffe and Smith (1932) Sugar and creatinine analyses were done twice in all experiments except those on T S, M W, and M M the deviation between the two analyses was slight, and the recorded figures are in every case averages of the two determinations

Our experiments are divisible into two groups, in the first of which (Table II) the effects of single doses of phlorizin on the urea, glucose, xylose, and creatinine clearances were followed Two control samples of blood and urine were collected prior to the injection of phlorizin, after a washout period of 20 to 50 minutes to clear the dead space of the kidneys, ureters and bladder, three more samples of urine and additional samples of blood were collected to enable us to observe the effects of phlorizin over a period of 40 to 60 minutes

The second group of experiments was designed to examine the effects of increasing doses of phlorizin on the glucose, xylose and sucrose clearances (Table III) The arrangement of these experiments was similar to those of the first group except for the omission of control periods

In the first group of experiments 11.8 mgm of phlorizin per kgm raised the glucose clearance for at least one hour to the level of the xylose clearance, and 65.2 mgm per kgm did not raise the former significantly above the latter In accordance with the observations previously published from this laboratory, we interpret these results to indicate that the last four individuals in Table II were "completely phlorizinized," i.e., the tubular reabsorption of glucose was completely blocked

Simultaneous xylose, glucose, urea and creatinine clearances in man following intravenous administration of phlorizin

Subject	Phlo rizin	Sur- face area	Total con- current time	Urine flow per minute	UV P/SA				Creatinine Xylose	Urea Xylose	Glucose Xylose	
					Xy- lose	Glucose	Crea- tinine	Urea				
T S ♂	mgm per kilo 2 04	sq m 1 51	35	1 30	20 4		35 9	14 6	1 76	72		
			54	95	17 9		31 0	11 6	1 73	65		
			79	88	14 1		26 0	9 8	1 84	69		
			105	Washout period								
			141	2 17	22 6	13 0	33 2	15 0	1 47	66	58	
			183	1 10	15 9	4 2	23 0	9 6	1 45	60	26	
M W ♀	4 12	1 18	21	1 00	53 0		104 3	38 4	1 97	72		
			37	1 50	64 7		128 5	49 1	1 99	76		
			80	Washout period								
			99	4 60	55 0	35 2	106 0	46 1	1 96	84	64	
			117	5 55	59 0	36 6	113 5	48 2	1 92	82	62	
			138	5 76	59 1	30 8	122 4	47 1	2 07	80	52	
M M ♀	6 07	1 52	31	3 20	18 4		43 2	14 5	2 35	79		
			63	3 20	34 3		78 5	26 5	2 29	77		
			90	Washout period								
			121	2 60	30 8	31 0	65 1	26 8	2 11	87	1 01	
			150	3 60	37 6	34 6	86 4	32 5	2 30	86	92	
			179	3 10	36 5	15 3	83 2	30 2	2 28	83	42	
A H ♀	11 8	1 27	18	12 70	58 0		106 7	44 0	1 84	76		
			74	Washout period								
			94	3 20	49 1	50 3	86 2	38 8	1 76	79	1 02	
			114	3 10	52 2	55 2	93 6	40 3	1 79	77	1 06	
			139	2 90	55 4	57 6	94 0	40 8	1 70	74	1 04	
M S ♀	15 7	1 39	25	1 60	53 5		96 3	41 7	1 80	78		
			72	Washout period								
			90	1 67	43 9	45 4	75 5	31 0	1 72	71	1 03	
			104	1 63	42 3	44 2	73 0	30 7	1 73	73	1 04	
			130	1 75	48 6	48 3	81 2	34 3	1 67	71	99	
C S ♀	20 4	1 47	21	7 30	58 6		109 5	34 3	1 87	58		
			41	2 60	60 8		112 3	35 4	1 85	58		
			74	Washout period								
			94	2 37	52 4	54 0	105 5	30 6	2 01	58	1 03	
			115	2 25	55 4	56 0	108 5	33 1	1 96	60	1 01	
			139	2 26	58 0	56 8	110 9	34 9	1 91	60	98	
N O ♀	65 2	1 60	20	9 70	43 0		76 3	33 1	1 77	77		
			44	6 62	41 4		82 5	34 3	1 99	83		
			91	Washout period								
			106	2 08	30 8	33 4	46 3	20 0	1 50	65	1 08	
			126	2 10	32 9	34 3	49 9	20 7	1 52	63	1 04	
			145	1 94	30 2	32 6	48 6	19 4	1 61	64	1 08	

TABLE III

Simultaneous xylose sucrose and glucose clearances in man following intravenous administration of phlorizin

Subject	Phlorizin	Surface area	Total con current time	Urine flow per minute	UV P/S.A.			Glucose Xylose	Sucrose Xylose
					Xylose	Glucose	Sucrose		
	<i>mgm per kgm</i>	<i>sq m</i>	<i>minutes</i>	<i>cc</i>					
A V ♀	21.7	1.58	18	6.64	57.1	45.8	56.0	0.80	0.98
			39	5.38	58.6	48.0	54.4	0.82	0.93
			60	4.41	50.6	43.2	48.5	0.85	0.96
			80	4.20	49.3	36.1	47.4	0.73	0.96
F M ♀	29.7	1.32	19	4.30	55.7	50.9	53.8	0.91	0.97
			39	3.95	55.4	55.4	53.6	1.00	.97
			64	3.48	53.3	54.1	54.3	1.02	1.02
V T ♀	41.9	1.59	31	4.90	46.8	41.0	45.0	0.88	0.96
			51	3.80	37.8	34.7	35.6	.92	.94
			75	5.79	48.4	45.5	48.4	.94	1.00
W M ♂	45.3	1.49	20	4.02	42.0	39.8	42.0	0.95	1.00
			43	3.97	47.0	45.9	46.1	0.98	.98
			66	4.09	49.5	48.1	50.0	0.97	1.01
			87	3.77	47.8	48.1	47.4	1.01	.99
F M ♀	59.4	1.32	29	4.30	55.1	56.0	58.6	1.02	1.06
			54	3.56	57.2	57.7	61.4	1.01	1.07
			82	2.86	46.2	44.8	48.5	.97	1.05
			104	2.18	44.3	44.0	45.7	.99	1.03

In the experiments listed in Table III, in which the comparison of xylose and sucrose was made, 21.7 mgm per kgm were apparently inadequate to produce complete glycosuria, but the variations in the glucose xylose ratio after larger doses must be considered to be within the experimental error. Again we pushed on to large doses to see if any one of the three sugars might be preferentially affected, but we find no evidence that such is the case. If xylose or sucrose were reabsorbed by the renal tubules, it might be imagined that a small dose of phlorizin would bring the glucose clearance up to the xylose clearance, while larger doses would raise the former definitely above the latter. To the contrary, it would seem that once the reabsorption of glucose is completely blocked, the simultaneous glucose, xylose and sucrose clearances remain identical within the experimental error, regardless of the quantity of phlorizin administered. In view of this fact, and in view of the further facts that the xylose clearance is not raised significantly in respect to the urea clearance, nor in respect to its own control level,¹ by the

¹ The single exception to this statement occurred in T S, who received the smallest dose of phlorizin given.

administration of phlorizin, we conclude that in man, as in the dog, the xylose (or sucrose) clearance is a measure of glomerular filtration (*cf* Jolliffe, Shannon and Smith, 1932)

Since, by the above interpretation, "complete phlorization" consists of a change in renal activity whereby all the glucose filtered at the glomeruli is allowed to pass into the urine, and since the term "complete phlorization" is open to several objections, we prefer to designate this condition as "complete glycuressis," in line with the use of this term as suggested by Benedict, Osterberg and Neuwirth (1918) to designate the increased rate of excretion of glucose, rather than the mere presence of this substance, in the urine. Thus, we are able to speak of complete glycuressis, transient glycuressis and recovery from glycuressis, a more flexible usage than is possible with the older term.

The fact that the urea clearance is consistently lower than the xylose clearance is uninterpretable at the present time. The urea-xylose ratios observed throughout this work confirm in general the observation of Jolliffe and Chasis (1933) and Keith, Power and Peterson (urea-sucrose, 1933) on man, and our own observations on the dog.

With regard to the effect of phlorizin on the creatinine clearance in man it will be noted that the administration of the drug resulted in a drop in the creatinine-xylose ratio only in subjects T S and N O. Since equally large variations in this ratio have been observed during the course of single experiments in man (unpublished details of Jolliffe and Chasis, 1933), we cannot attach particular significance to this result, and we conclude that larger doses of phlorizin are required to reduce the creatinine clearance to the level of the xylose clearance (i.e. to depress completely the tubular secretion of creatinine) than are required to produce complete glycuressis. It would be of interest to know if complete depression of creatinine secretion could be obtained in man as in the dog, but we decided that the question did not justify the administration of intravenous doses larger than those we have given.

In conclusion, it may be noted that the glycuressis induced in man by phlorizin is transient, as is the case in the dog. All our subjects were tested for glycosuria at intervals following the formal observations, and found to be essentially sugar-free within 24 hours.

SUMMARY

The minimum intravenous dose of phlorizin required in man to produce complete phlorization (i.e., to raise the glucose clearance to xylose clearance) was found to be in the neighborhood of 10 to 20 mgm per kgm.

Sixty-five mgm per kgm did not raise the glucose clearance above the xylose clearance, and 59.4 mgm per kgm did not cause deviation between the xylose and sucrose clearances. Neither did the phlorizin

cause a significant rise in the xylose clearance as compared to control periods taken just before the administration of the drug

In the largest dose which we have given (650 mgm per kgm) the drug exerted no significant depressive action on the ratio of creatinine xylose clearance

BIBLIOGRAPHY

- Achard, C, and Delamare, V, *Compt rend Soc de biol*, 1899, li, 48 La glycosurie phloridzique et l'exploration des fonctions rénales
- Allard E, *Arch f exper Path u Pharmacol*, 1907, lvi, 241 Untersuchungen über die Harnabsonderung bei Abflussschwerung
- Benedict, S R and Lewis, R C, *Proc Soc Exper Biol and Med*, 1914 xi 134 The Influence of Induced Diabetes on Malignant Tumors (Including a Report of a Case of Human Phlorhizin Glycosuria)
- Benedict S R Osterberg, E, and Neuwirth, I, *J Biol Chem*, 1918 xxiv, 217 Studies in Carbohydrate Metabolism II A Study of the Urinary Sugar Excretion in Two Normal Men
- Casper L, and Richter P F, *Berl klin Wchnschr*, 1900, xxxvii, 643 Ueber funktionelle Nierendiagnostik
- Chabanier, H, and Lobo Onell, C *Compt rend Soc de biol*, 1913 lxxiv 681 Indépendance de la sécrétion uréique et de la sécrétion glucosique provoquée par la phloridzine
- von Coetsem C A *Bull Soc Méd Gand*, 1836, May 3, p 90, June 7, p 108 (Cited by Delamare)
- Delamare, V, La glycosurie phloridzique son application à l'exploration clinique des fonctions rénales Thèse de Paris 1899
- Deuel, H J, Jr and Chambers W H, *J Biol Chem* 1925, lxxv, 7 The Rate of Elimination of Ingested Sugars in Phlorhizin Diabetes
- Dünner, L, and Kronenberger, F, *Klin Wchnschr*, 1930 ix, 1256 Ueber den Einfluss des Phlorrhizins auf die Magensekretion
- Dünner, L, and Mecklenburg, M, *Ztschr f d ges exper Med*, 1925, xlv, 518 Klinisch experimentelle Untersuchungen mit Phlorizin
- Grote, L R, *Verhandl d deutsch Gesellsch f inn Med*, 1921, xxxiii 291 Inkretorischer Einfluss auf die Phlorrhizinglukosurie
- Hanegraeff *Essai sur la phloridzine dans le traitement de la fièvre intermittente* Antwerp 1837 (Cited by Delamare)
- Hetényi S, *Biochem Ztschr*, 1922, cxxix, 183 Die Wirkung des Phlorrhizins auf den Blutzucker bei diffusen doppelseitigen hämatogenen Nieren erkrankungen Ein Betrag zur Frage der Phlorrhizinwirkung
- Jolliffe N and Chasis H, *Am J Physiol*, 1933 civ, 677 The Filtration and Secretion of Exogenous Creatinine in Man
- Jolliffe N Shannon, J A and Smith, H W, *Am J Physiol* 1932, c 301 The Excretion of Urine in the Dog I The Use of Non metabolized Sugars in the Measurement of Glomerular Filtrate
- Kamnitzer and Joseph, *Therapie d Gegenw*, 1921, xxiii, 321 Zur biologischen Diagnostik der Schwangerschaft
- Kamnitzer and Joseph *Med Klin*, 1924, xx, 453 Weitere Erfahrungen mit Maturin als Diagnostikum der Frühgravidität gleichzeitig experimentelle Untersuchungen über die Wirkung des Phlorrhizins
- Keith, N M, Power, M H, and Peterson, R D, *Am J Physiol (Proc)*, 1933, cv, 60 The Renal Excretion of Sucrose when Injected Intravenously in Man

- de Koninck, L , Bull Soc Méd Gand , 1836, pp 75 and 110 Observations sur les propriétés fébrifuges de la phloridzine
- de Koninck, L , Ann Soc Sci Méd et Nat Bruxelles, 1836, p 1 Mémoire sur les propriétés et l'analyse de la phloridzine (Cited by Delamare)
- Klemperer, G , Verhandl d deutsch Gesellsch f inn Med , 1896, May 18 Ueber regulatorische Glykosurie und renalen Diabetes (Cited by Delamare)
- Korte, J , Phlorhizindiabetes insbesondere beim Menschen Dissert, Halle, 1896
- Magnus-Levy, A , Deutsche med Wchnschr , 1896, xxi, Vereins-Beilage No 1, 202 (V) Discussion of Klemperer's paper Ueber regulatorische Glykosurie und renalen Diabetes
- Mareska, D B J , Bull Soc Méd Gand , 1836, May 3 (Cited by Delamare)
- von Mering, J , Centralbl Med Wiss , 1885, xxiii, 531 Ueber kunstlichen Diabetes
- von Mering, J , Verhandl Cong inn Med , 1886, v, 185 Ueber experimentellen Diabetes
- Idem, Verhandl Cong inn Med , 1887, vi, 349 Ueber Diabetes mellitus
- Idem, Ztschr klin Med , 1889, xvi, 431 Ueber Diabetes mellitus II
- de Muynck, J J , Bull Soc Méd Gand , 1836, June 3 (Cited by Delamare)
- Pietkiewicz, T , Inaug Dissert , Dorpat , 1869 (Cited by v Mering, 1889)
- Rosenberg, M , Klin Wchnschr , 1923, ii, 342 Über die wechselseitige Beeinflussung von Blut- und Harnzucker durch Phlorizin bei Zucker- und Nierenkranken
- Shannon, J A , Jolliffe, N , and Smith, H W , Am J Physiol , 1932, ci, 625 The Excretion of Urine in the Dog IV The Effect of Maintenance Diet, Feeding, etc , upon the Quantity of Glomerular Filtrate
- Tanaka, T , Arch f exp Path u Pharmacol , 1908, lx, 1 Über die Viskosität, Gefrierpunkterniedrigung, Acidität und elektrische Leitfähigkeit des normalen Harns und des Phloridzindiabetes-harns von Japanern

AMINO NITROGEN CHANGES OF THE BLOOD IN NEPHRITIS

By ESBEN KIRK

(From the Hospital of the Rockefeller Institute for Medical Research, New York City)

(Received for publication July 25 1933)

Amino nitrogen and peptide nitrogen content of the blood in nephritic patients have been frequently determined since the introduction of suitable micro methods. Gasometric determinations of amino nitrogen and peptide nitrogen of plasma and red blood cells separately have, however, not yet been done.

That colorimetric analyses of the same blood samples give lower results for amino acid nitrogen than the gasometric method or the formaldehyde titration, and that colorimetric recovery of amino acids added to blood is incomplete, have been reported by Van Slyke and Kirk (18). The use of different analytical methods may accordingly explain the conflicting results reported in the literature. Thus most observers using the colorimetric method of Folin (Berglund (2), Greene, Sandiford and Ross (6), Schmidt (16), Witts (20), Feinblatt and Shapiro (5)) found no deviation from the normal in series of patients with nephritis, including several cases of uremia. Slightly or moderately increased values of amino nitrogen in uremia were reported with the colorimetric method by Wowski and Gelbird (21), and by Looney (11) in an individual case of mercuric chloride poisoning shortly before death. Extensive studies have been made in recent years in Volhard's clinic by Becher and Herrmann (1) with a modification of Folin's colorimetric technique, increase of free amino nitrogen of whole blood was frequently observed in patients with severe impairment of the kidney function. Only in a single case, however, did the method used indicate values exceeding 10 milligrams per cent.

Most of the work done with the gasometric method is of earlier date. Using various protein precipitants Bock (3), Okada and Hayashi (14), and Desqueyroux (4), all found increased amino nitrogen of the blood in cases of advanced nephritis. In nephritic toxemias of pregnancy, on the other hand, Losee and Van Slyke (12), and Morse (13) observed no marked deviation from the normal.

Quantitative determination of peptide nitrogen of blood was first made with exact methods by Hiller and Van Slyke in 1922 (7) by gasometric determination of blood filtrates before and after hydrolysis with hydrochloric acid. A similar procedure of acid hydrolysis has been used by different authors in studies of the peptide nitrogen in nephritis,

but in most cases other methods were substituted for the gasometric to determine the amino nitrogen before and after hydrolysis. When comparing results both the method of amino nitrogen determination and the protein precipitant used should be considered. Hulse and Strauss (9), using the formaldehyde titration method of Sørensen, and Hulse and Franke (8), using the gasometric method, observed high values for peptide nitrogen in nephritic patients with hypertension. Jackson, Sherwood and Moore (10), Becher and Herrmann (1), and Schlossmann (15), using the colorimetric method, were, however, unable to find any relation between the blood pressure and the content of peptide nitrogen in the blood in nephritis. A definite increase in peptide nitrogen was frequently seen by Becher and Herrmann (1) in cases of severe renal insufficiency, the peptide nitrogen even occasionally exceeding the concentration of free amino nitrogen, increase of peptide nitrogen with normal amino nitrogen values was sometimes observed.

METHODS

For separate analyses of cells and plasma, oxalated blood was centrifuged till constant volume of the red blood cells was obtained, plasma was then syphoned off and the intermediate layer of plasma and cells discarded. Samples of red blood cells were measured with a calibrated pipette "to contain."

Urea was transformed into ammonia by addition of a urease solution and phosphate buffer as described by Van Slyke (17). The proteins of plasma or whole blood were then precipitated by the Folin-Wu method. To plasma or whole blood one volume of 10 per cent sodium tungstate and one volume of $2/3$ N sulfuric acid were added, and the mixture was diluted with water to 10 times the original volume of the sample. For precipitation of the proteins of the red blood corpuscles two volumes of 10 per cent sodium tungstate and two volumes of $2/3$ N sulfuric acid were used for each volume of cells, but the sample was finally, as in precipitation of plasma and whole blood, made up to 10 times its original volume with water. Five cc of filtrate, therefore, represented 0.5 cc of plasma, red blood corpuscles or whole blood. Usually, however, the filtrate from the red blood corpuscles was again diluted with two volumes of distilled water to obtain sufficient material for analysis. Because of the high amino nitrogen content of the red blood cells the dilution did not diminish the amino nitrogen readings enough to interfere with accuracy.

The ammonia of the filtrates, formed by the splitting of the urea with urease, was removed by boiling with milk of magnesia and amino nitrogen afterwards determined by the manometric method of Van Slyke (17).

For determination of peptide nitrogen the urea-and-ammonia-free filtrates were heated on a water bath for 24 hours with equal volumes of concentrated hydrochloric acid. After evaporation of the acid in an open dish each residue was neutralized with a few drops of a saturated solution of sodium acetate and finally made up to volume (7). It was found unnecessary to subject the hydrolyzed samples again to boiling with milk of magnesia before the amino acid determination, as identical results were obtained with and without such treatment.

For blank analyses a urease solution was prepared, and precipitated, and

the filtrate was diluted as described by Van Slyke (17 p 442), or Peters and Van Slyke (22). The amino nitrogen content of the filtrate was determined before and after hydrolysis, to obtain the c correction. Separate blanks were determined for analyses of plasma and red blood corpuscles.

The normal magnitude and constancy of results obtained with the above procedure is illustrated by Table I. It gives the results from two samples of the same blood which were centrifuged and analysed separately. The sample of plasma filtrate represented 0.5 cc of plasma, the sample of filtrate of red blood cells 0.167 cc of corpuscles.

TABLE I
Showing reproducibility of measurements

	Milligrams per cent amino nitrogen	
	I	II
Plasma unhydrolyzed	4.10	4.07
Plasma hydrolyzed	4.10	4.03
R B C unhydrolyzed	12.85	12.91
R B C, hydrolyzed	18.89	18.03

Studies of amino nitrogen and peptide nitrogen of the blood were made in seven uremic patients. (See Tables II and III.)

TABLE II
Amino nitrogen in whole blood during development of uremic coma
Case No 1, M J Hospital No 7855

Date	Amino nitrogen per 100 cc.			Whole blood nonprotein nitrogen per 100 cc.	Whole blood urea nitrogen per 100 cc.	Urea clearance	Condition
	Plasma	Cells	Whole blood				
1931	mgm	mgm	mgm	mgm	mgm	per cent of mean normal	
September 27			18.0	148	118		Uremia
September 28			13.7	150	119	6	Uremia
October 13							
10 00 A M }			12.3	171	191		Uremia }
9 30 P M }			21.4	189	141		Coma }
October 29	28.8	36.7					Coma

CLINICAL NOTES ON UREMIC CASES¹

Case No 1 Hospital No 7855 M J, female, 33 years As a child had

¹ The nomenclature followed here for the different types and stages of Bright's disease is in general that used by Van Slyke Stillman et al (19). In addition, to distinguish the different conditions near and in coma, the following terms are used.

Terminal stage with nitrogen retention Nitrogen retention without clinical symptoms

Uremia Nitrogen retention with clinical symptoms, such as nausea and vomiting but with normal consciousness

Semi-comatose condition Consciousness greatly influenced, but patient still responds

Coma Patient unconscious and does not respond

TABLE III
Uremic cases 2, 3, 4, 5 and 6

Subject			Amino nitrogen per 100 cc						Blood urea nitrogen per 100 cc	Urea clearance	Condition
Initials and serial number	Hospital number	Date	Plasma		Cells		Peptide				
			Free	Peptide	Free	Peptide					
EH No 2	8166	1932 May 29 June 2	mgm	mgm	mgm	mgm	mgm	per cent of mean normal	2	Uremia Coma	
			23.4	-1.0	12.2	16.5					
			37.3	7.0	24.8	18.0					
JD No 3	8080	1932 June 29 July 7	16.8	-0.6	12.4	6.3	Uremia Uremia				
			12.3	1.0	18.4	17.0					
EB No 4	7884	1931 November 5 November 6 December 21	5.9	1.9	13.5	12.0	Good				
			6.9	-0.2	15.3	2.9					
			6.5	0.4	13.1						
		1932 May 26 June 1 August 29 September 2	6.5		14.1	8	140	Coma Died 48 hours later			
			6.6		12.4						
			21.3	-5.1	12.9				9.5		

TABLE III (continued)

Subject			Amino nitrogen per 100 cc.				Blood urea nitrogen per 100 cc.	Urea clearance	Condition
Initials and serial number	Hospital number	Date	Plasma		Cells				
			Free	Peptide	Free	Peptide			
			mgm.	mgm	mgm	mgm	mgm	per cent of means normal	
M W No 5	6473	1931							
		December 6	21 80	-2 29	19 24	10 56	163	7 6	Coma Intravenous glucose
		December 10	22 40	-1 99	16 25	9 85	143		Still comatose but condition improved
		December 15	10 24	92	11 80	8 26		7 2	Condition improved
		December 16	7 18	1 33	12 90	8 25			Condition good
		December 17	5 82	1 66	13 94	7 00	151		Ascites developing
		December 21	21 12	12	14 32	8 08	152		Fever (till December 24)
		December 22	7 29	1 27	14 70	7 28	157	7 2	Condition good
		December 26	10 20	1 20	10 67	7 32			Condition good
		December 29	8 19				127	8 4	Condition good
		1932							Condition good
		January 1	5 95	10 21	16 54	7 19	126		Condition good
		January 5	6 93	33	14 45	13 54	118		Condition good
		January 11	11 52	-	17 16 07	9 36	91	7 1	Condition good
		May 1	14 22	-	15 51	9 27	95		Condition good
		September 21						3 4	Coma
		September 26	28 58	2 42	7 10	9 38	239		Coma
		September 27	28 20	-1 58	9 80	2 81	295		

TABLE III (continued)

Subject			Amino nitrogen per 100 cc					Blood urea nitrogen per 100 cc	Urea clearance	Condition
Initials and serial number	Hospital number	Date	Plasma		Cells					
			Free	Peptide	Free	Peptide	Free			
M R No 6	7872	1931 November 24 December 3 1933 May 21 June 13 June 14 9 30 A M 9 30 P M 11 00 P M June 15 6 45 A M 11 45 A M	mgm	mgm	mgm	mgm	mgm	<i>per cent of mean normal</i> 26 22 7 <		

hemorrhagic Bright's disease following tonsillitis. After several years of active symptoms the patient went into a latent stage, but the renal lesion became active again during a pregnancy, necessitating abortion. In the following period of ten years exhibited hypertension and proteinuria. Two weeks before admission vomiting, edema of feet and dyspnea developed. On admission (September 25, 1931) was in uremia.

Case No 2 Hospital No 8166 E H, female, 21 years. Two years before admission had hematuria and hypertension. Visual disturbances, edema of ankles, vomiting and diarrhea had been present in the last weeks. On admission (May 28, 1932) was in uremia.

Case No 3 Hospital No 8080 J D, male, 30 years. Ten months before admission developed tonsillitis, proteinuria and hypertension, followed by edema and ascites. In the last days had had severe nose bleedings. On admission (June 28, 1932) was in uremia.

Case No 4 Hospital No 7884 E B, male, 29 years. Nine years before admission had had respiratory infection with hematuria. Recovered into a latent stage with proteinuria as only symptom. In the last six months had had hypertension and severe headache. The patient was observed for several months during the chronic active stage and during the last weeks of terminal uremia.

Case No 5 Hospital No 6473 M W, male, 15 years. Three and a half years before admission developed generalized edema with proteinuria and reduced kidney function following an attack of upper respiratory infection. In the following period gave evidence of activity in the kidney lesion (microscopic hematuria). Immediately before admission (December 3, 1931) suffered from abnormal sleepiness and attacks of convulsions.

Case No 6 Hospital No 7872 M R, female, 19 years. Four months before first admission had edema of the legs. On admission (October 21, 1931) macroscopic hematuria, hypertension and reduced kidney function were present. In the following 18 months the nephritis progressed through the chronic active to the terminal stage. Two weeks before last admission (May 4, 1933) reduction of vision and occasional cardiac decompensation were noted. Death occurred on June 15, 1933, after two days of a semicomatose condition.

Case No 7 Hospital No 8342 M C, male, 19 years. Four years before admission developed proteinuria (and hematuria?) following mastoiditis. Went into a latent stage of hemorrhagic Bright's disease. Three weeks before admission had had inflammation of the mandibular region caused by hemolytic streptococci. In relation to this infection there occurred acute exacerbation of the nephritis with oliguria, gross nitrogen retention and vomiting. On admission (November 25, 1932) was in acute uremia, markedly dehydrated. During the first days in the hospital was at intervals extremely drowsy, but not comatose.

Following treatment of the dehydration the patient made a quick recovery to an excellent general condition. The activity of the renal lesion persisted however, and the kidney function never exceeded 10 per cent of the average normal urea clearance.

Amino nitrogen determinations of plasma were made daily for a period of about three months. The data are presented in Figure 1. The highest value, 38.7 mgm of amino nitrogen per 100 cc of plasma, was observed on November 29, 1932 at a time when the patient was very drowsy.

TABLE IV
Amino nitrogen and peptide nitrogen content of blood of normal individuals and non-uremic nephritic patients

Subject	Date	Amino nitrogen in plasma		Amino nitrogen in red blood cells		Urea clearance	Diagnosis
		Free mgm per 100 cc	Peptide mgm per 100 cc	Free mgm per 100 cc	Peptide mgm per 100 cc		
A A	September 27, 1932	5 20	— 54	11 10	6 95	per cent of mean normal	Normal
S S	August 7, 1932	6 56	55	11 09	6 37		Normal
E K	December 10, 1931	5 29	41	18 07	5 01		Normal
Same	January 21, 1932	4 48	— 16	13 14	7 27		
Same	April 15, 1932	5 51	17	20 51	9 32		
M S	September 28, 1932	4 33	— 06	7 99	5 12		Normal
J M	September 28, 1932	4 93	— 30	10 15	4 85		Normal
W M	September 29, 1932	4 14	19	8 26	3 95		Normal
Hospital Number 7943	January 4, 1932	5 46	23	12 44	7 01	78	Hemorrhagic Bright's disease initial
7986	April 7, 1932	5 93	37	14 54	4 12	30	Same
7828	November 25, 1931	5 12	— 72	10 21	5 98	109	Same
7872	November 24, 1931	5 57	— 18	12 24	9 28	26	Same
Same	December 3, 1931	11 63	— 1 62	13 80	5 05	22	Same
7450	November 19, 1931	4 45	— 15	10 98	6 82	92	Hemorrhagic Bright's disease active
7842	January 6, 1932	4 90	— 32	8 33	6 53	44	Same
7884	November 5, 1931	5 87	1 86	13 46	11 97	60	Same
Same	November 6, 1931	6 92	— 22	15 29		60	Same
Same	December 21, 1931	6 48	44	13 09	2 89	90	Same
7938	December 17, 1931	6 49	19	8 89	9 57	88	Hemorrhagic Bright's disease latent
7874	November 19, 1931	5 08	— 15	13 60	5 64	161	

TABLE IV (continued)

Subject	Date	Amino nitrogen in plasma		Amino nitrogen in red blood cells		Urea clearance	Diagnosis
		Free	Peptide	Free	Peptide		
7923	December 2 1931	mgm per 100 cc 7 83	mgm per 100 cc. 1 01	mgm per 100 cc 13 55	mgm per 100 cc. 5 55	per cent of mean normal 17	Hemorrhagic Bright's disease terminal
Same	December 10 1931	7 84	— 23	17 74	6 86	15	Same
Same	April 14, 1932	10 45	33	19 69	10 55	10	Same
7867	October 24 1931	7 83		15 85		19	
8101	April 23, 1932	5 00	47	16 40	6 47	18	Myeloma Bence- Jones proteinuria
7922	January 3, 1932	8 21	— 1 47	12 56	5 79	36	Degenerative Bright's disease same (Eti- ology pregnancy)
7905	November 13 1931	4 90	— 26	13 56		97	Same
Same	November 30 1931	4 05	16	12 11	6 07	90	
7963	January 25 1932	5 58		16 07		14	Arteriosclerotic Bright's disease
7837	December 29 1931	4 08	01	12 88	5 58	54	Same
8063	April 8 1932	4 71	1 43	13 94	1 94	35	Same
7876	November 9 1932	7 78	— 54	13 69	5 81	61	Hemorrhagic Bright's disease with ne- phrotic component
Same	November 10 1931	6 88		13 55	7 46	61	Same
Same	November 24 1931	8 04	— 57	12 73	8 50	57	Same
Same	December 3, 1931	5 66	— 1 66	10 25	6 07	58	Same

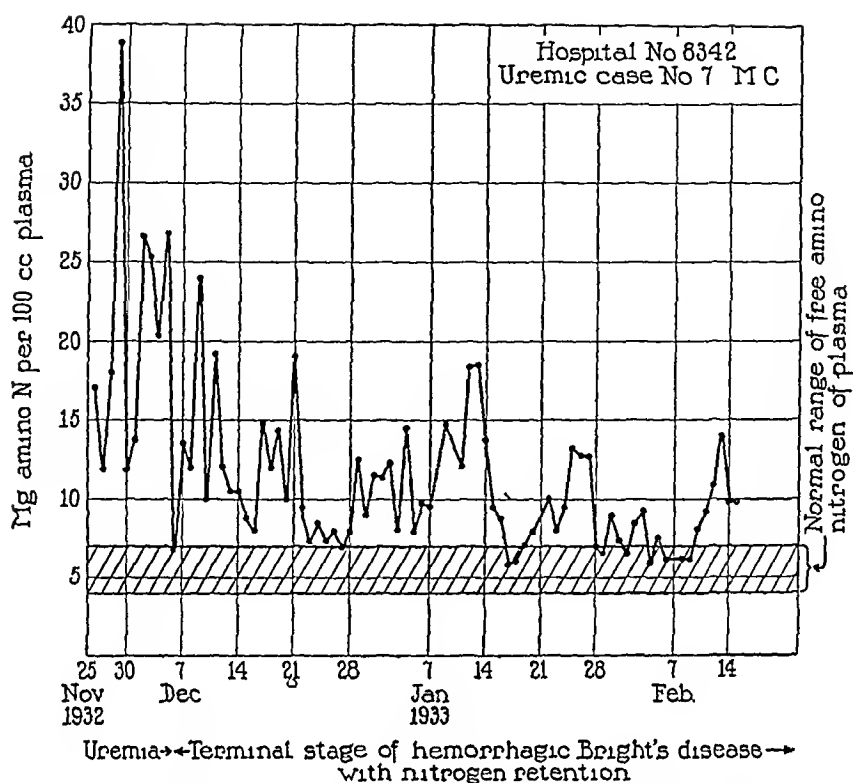


FIG 1 AMINO NITROGEN CONTENT OF BLOOD PLASMA DURING AND AFTER RECOVERY FROM ACUTE UREMIA

SUMMARY OF RESULTS

In cases of chronic and acute nephritis with more than 40 per cent of normal urea clearance the plasma amino nitrogen content, determined gasometrically, was found normal, 4 to 6.5 mgm per 100 cc

In more advanced cases, but without uremic symptoms, the plasma amino nitrogen was sometimes normal and sometimes increased to 8 to 10 mgm per 100 cc

In the pre-coma stage, the plasma amino nitrogen was variable, and might change in 24 hours from practically normal to over 20 mgm per 100 cc

In uremic coma, the plasma amino nitrogen was found uniformly high, from 2 to 7-fold normal

The amino nitrogen fluctuations in the plasma were more marked than in the cells, and more regularly related to the condition of the patients. Significant variations in the plasma peptide nitrogen were not observed

CONCLUSIONS

Elevation of plasma amino nitrogen tends to be more frequent as renal disease becomes more advanced. The correlation between fall in

renal function and rise in plasma amino nitrogen is irregular, however, in some cases the renal clearance approaches the coma level (about 5 per cent of normal clearance) before rise in plasma amino nitrogen occurs. It appears probable, therefore, that the rise in amino acid content is not directly due to renal failure, but to a breakdown in the mechanism for metabolism of the amino acids occurring elsewhere as part of the general debacle.

BIBLIOGRAPHY

- 1 Becher, E, and Herrmann, E, *Deutsches Arch f klin Med* 1932, cxviii, 23 Der freie und gebundene Aminostickstoff im enterweissten Blut und Gewebe unter normalen und pathologischen Verhältnissen IV Der freie und gebundene Amino N im enterweissten Blut bei essentieller Hypertonie und Nierenkrankheiten
- 2 Berglund H, J A M A, 1922, lxxix, 1375 Nitrogen Retention in Chronic Interstitial Nephritis and its Significance
- 3 Bock, J C, *J Biol Chem* 1917, cxix, 191 The Amino acid Nitrogen Content of the Blood of Various Species
- 4 Desqueyroux, J, *Ann de Méd*, 1923, xiii, 20 Recherches cliniques sur l'acide aminé
- 5 Feinblatt H M, and Shapiro I, *Arch Int Med*, 1924, cxliiv, 690 The Amino acid Content of Blood in Various Pathological Conditions An Analysis of One Hundred and Sixty Determinations on One Hundred and Twenty Persons
- 6 Greene, C H, Sandiford, K, and Ross, H, *J Biol Chem*, 1924, lvi, 845 The Amino acid Content of the Blood in Normal and Pathologic Conditions
- 7 Hiller A, and Van Slyke, D D *J Biol Chem*, 1922, lvi, 253 A Study of Certain Protein Precipitants
- 8 Hülse, W, and Franke K, *Arch f exp Path u Pharmacol*, 1929, cxliii, 257 Weitere Untersuchungen zum Chemismus der nephritischen Blutdrucksteigerung
- 9 Hulse, W, and Strauss H *Ztschr f d ges exper Med* 1924, xxxix, 426 Zur Frage der Blutdrucksteigerung V Über die Wirkung höherer Eiweisspaltprodukte auf den Blutdruck und ihr Vorkommen im Blute bei hypertensischen Nierenkrankheiten
- 10 Jackson H, Jr Sherwood, D W, and Moore O J, *J Biol Chem*, 1927, lxxiv, 231 The Blood Peptide Nitrogen in Arterial Hypertension
- 11 Looney J M *J Biol Chem* 1926, lxx, 513 Blood Changes in Acute Mercuric Chloride Poisoning
- 12 Losee, J R, and Van Slyke D D, *Am J M Sc*, 1917, cliii, 94 The Toxemias of Pregnancy
- 13 Morse A Johns Hopkins Hosp Bull 1917 cxviii 199 The Amino acid Nitrogen of the Blood in Cases of Normal and Complicated Pregnancy and also in the New born Infant
- 14 Okada S, and Hayashi, T, *J Biol Chem* 1922, li, 121 Studies on the Amino acid Nitrogen Content of the Blood
- 15 Schlossmann, H, *Ztschr f d ges exper Med*, 1925, xlvii, 487 Der Gehalt des Blutes an Aminosäuren und Polypeptiden in Schwangerschaft Geburt und Wochenbett mit besonderer Berücksichtigung der Schwangerschaftstoxämien
- 16 Schmidt, E G, *Arch Int Med* 1929, xlv, 351 The Amino acid Content of the Blood in Health and in Disease

- 17 Van Slyke, D D , J Biol Chem , 1929, lxxxi, 425 Manometric Determination of Primary Amino Nitrogen and its Application to Blood Analysis
- 18 Van Slyke, D D , and Kirk, E , J Biol Chem , 1933 (In Press) Comparison of Gasometric, Colorimetric, and Titrimetric Determinations of Amino Nitrogen in Blood and Urine
- 19 Van Slyke, D D , Stillman, E , Møller, E , Ehrich, W , McIntosh, J F , Leiter, L , Mackay, E M , Hannon, R , Moore, N S , and Johnston, C , Medicine, 1930, ix, 257 Observations on the Courses of Different Types of Bright's Disease, and on the Resultant Changes in Renal Anatomy
- 20 Witte, L , Quart J Med , 1929, xxi, 477 Observations on the Metabolism of Amino-acids in Health and Disease
- 21 Wowski, M , and Gelbird, J , Ztschr f d ges exper Med , 1926, li, 518 Über die Rolle der Leber im Aminosäurenstoffwechsel
- 22 Peters, J P , and Van Slyke, D D , Quantitative Clinical Chemistry II Methods Williams and Wilkins Co , Baltimore, 1932, p 397

A FORMULA AND NOMOGRAM FOR THE ESTIMATION OF THE OSMOTIC PRESSURE OF COLLOIDS FROM THE ALBUMIN AND TOTAL PROTEIN CONCENTRATIONS OF HUMAN BLOOD SERA

By HERBERT S. WELLS, JOHN B. YOUMANS, AND DAVID G. MILLER, JR.

(From the Departments of Physiology and Medicine, Vanderbilt University Medical School, Nashville)

(Received for publication July 31, 1933)

In connection with investigations to be reported subsequently the osmotic pressure of colloids and the albumin and total protein concentrations of 128 samples of human blood serum were determined. From these data, which cover a wide range of values, an empirical formula has been evolved which, it is believed, will enable the osmotic pressure to be more accurately estimated from the protein analyses of human sera than is the case with formulae now employed for this purpose (1, 2). A nomogram has been constructed to facilitate such computations. The simple, definitely linear relation found to exist between the albumin concentration and the specific osmotic pressure would indicate that the albumin-globulin ratio is not as reliable an index of the osmotic properties of serum as is at present often assumed.

It may justly be contended—at least on theoretical grounds—that the accuracy of most of the colloid osmotic pressure measurements previously carried out on human blood sera is open to serious question, either for the reason that the authors have failed to recognize the fundamental criteria of equilibrium upon which any good physical chemist would insist (3) or because of the failure to describe the methods employed in sufficient detail to enable one to judge as to whether such criteria have actually been fulfilled.

In a recent communication (4) it was emphasized that, for the accurate determination of the osmotic pressure of colloids, it is essential to observe certain rigid criteria of an osmotic equilibrium. Procedures were described by which these criteria of accuracy might be fulfilled, and a detailed description of the apparatus and methods has recently been published (5). By a strict adherence to such standards it has been possible, during the present investigation, to obtain from duplicate determinations on each serum pressure values which, in 95 per cent of the cases, do not differ by more than 12 mm. of water. No pressure reading has been considered to be accurate unless it has been possible to maintain the volume of the serum at a constant value, at this pressure

and at constant temperature, for a period of at least two hours—a period of observation sufficient, according to our tests, to insure the attainment of a definite equilibrium when the membranes are of the standard selected permeability. Consequently, it is believed that our figures actually refer to equilibrium pressures. The fact that the standard per cent deviation of the calculated from the observed values of the osmotic pressure is only ± 5 per cent leads us to believe, furthermore, that for routine clinical purposes, the method of calculation, which avoids the many difficulties inherent in the usual methods of direct determination, will provide a sufficiently reliable index of the water-retaining power of the serum. Such a conclusion is, however, to be considered as subject to the same qualifications as apply to all cases of indirect measurements, as for example, the estimation of the basal metabolic rate from the oxygen consumption. The possibilities of error in the use of the calculated figures will be discussed in later paragraphs.

METHODS

Protein analyses were carried out in duplicate by the macro-Kjeldahl method on samples drawn under oil without stasis. Globulin was separated by precipitation at 38°C with 22.5 per cent sodium sulphate according to the method of Howe (6). Nonprotein nitrogen was estimated by the micro-Kjeldahl-Nesslerization method, on oxalated plasma. The usual factor, 6.25, was used to convert protein nitrogen values to protein concentrations.

The osmotic pressure determinations were carried out in a constant temperature room at 20° to 22°C . Duplicate determinations were done in all but four instances. The collodion membranes used were standardized to a specific permeability (4) range of 20×10^{-8} to 40×10^{-8} . Equilibrium was usually attained in 1 to 3 hours, but a somewhat longer time was occasionally required. The details of the method and the criteria of osmotic equilibrium have been described elsewhere (4, 5). Occasional lapses of the vigilant attention to detail which is necessary for success in carrying out such determinations invariably led to gross errors which necessitated discarding the results. On the other hand, all data have been included for which definite evidence of gross errors of technic was lacking, regardless of the degree of variation between the duplicate determinations.

DATA

The blood samples were taken from 53 individuals, from 1 to 10 determinations being carried out on each person, often over periods of several months. Of these 53 persons 14 were laboratory workers, students, and other volunteers, all apparently in good health, 20 presented, at one time or another, and to various degrees, evidences of nutritional edema, 12 had heart disease, and 1 each suffered from the following

pathological conditions multiple xanthomatosis, chronic diarrhea, aplastic anemia, pellagra with chronic colitis, Banti's disease, nephrosis, syphilitic cirrhosis of the liver

The data are presented in Table I, arranged in order of increasing albumin concentrations. The 10 lowest albumin, total protein and osmotic pressure figures were obtained from a single individual, over a period of 5 months during the gradual decline associated with an advanced nephrosis. The 13 highest values were obtained from blood which had been concentrated by causing the subjects to stand in the erect posture for one hour or longer, in connection with experiments which are to be described elsewhere. The extreme range of the values encountered may be indicated by the following figures: albumin, per cent, 0.89 to 6.62, globulin, 1.66 to 6.48, total protein, 2.99 to 9.64, albumin globulin ratio, 0.42 to 2.80, osmotic pressure of colloids, 76 to 583 mm water, and specific osmotic pressure, 25.4 to 59.2 mm water. The figures for the albumin concentrations probably furnish the best index as to the distribution of the values. There were 10 albumin values below 2.5, 13 between 2.5 and 3.5, 50 between 3.51 and 4.5, 48 between 4.51 and 5.5, and 7 above 5.5.

ANALYSIS OF THE DATA

The specific osmotic pressure, S , was calculated for each case by dividing the value of the total osmotic pressure, P , measured in mm of water, by the concentration of total proteins, C , measured in grams per 100 cc. Values for the specific pressures were plotted on a three-dimensional graph against the corresponding values of the albumin and of the globulin concentrations. This was done by driving wires, sharpened at one end, into the drawing board on which the axes of albumin and globulin concentrations (A and G) were laid off on cross section paper. Each value of S was thus represented by the height of a wire and the point through which the wire was driven represented the corresponding A and G values. The surface produced by the tips of the wires was seen to be fairly regular, but contrary to our earlier expectations it did not show any obvious relation between the specific osmotic pressure and the globulin concentration or A/G ratio. The formula of Govaerts (1) implies the assumption that the specific pressure is determined primarily by the A/G ratio. According to his concept the value of S will fall with increasing values of G for sera having a constant value of A . Govaerts also assumes that the total protein concentration, C , is without effect on the value of S in the case of sera which have the same A/G ratio. In our series there is an obvious and regular relation between C and S and it was apparent from inspection of the wire model, that the increasing values of S , as C increased, were due entirely to the increase in A . The relation between the specific pressure and the albumin was

TABLE I

Data arranged in ascending order of albumin concentrations

Case number	Albumin	Specific pressure observed	Specific pressure calculated	Total protein	Osmotic pressure observed	Osmotic pressure calculated	Variation of calculated from observed pressure	Maximum experimental error	Unexplained variations
	<i>grams per 100 cc</i>	<i>mm H₂O</i>	<i>mm H₂O</i>	<i>grams per 100 cc</i>	<i>mm H₂O</i>	<i>mm H₂O</i>	<i>mm H₂O</i>	<i>mm H₂O</i>	<i>mm H₂O</i>
37	0.89	25.4	26.7	2.99	76	80	+4	±21	
37	1.25	30.9	28.8	3.10	96	89	-7	±22	
37	1.46	29.3	30.0	3.35	98	101	+3	±22	
37	1.75	30.1	31.7	3.56	107	113	+6	±23	
37	1.93	31.2	32.8	3.59	112	118	+6	±23	
37	1.93	30.4	32.8	3.71	113	122	+9	±23	
37	1.94	31.3	32.8	3.89	122	128	+6	±23	
37	2.28	38.6	34.9	4.09	158	143	-15	±24	
37	2.41	34.6	35.6	4.16	144	148	+4	±24	
37	2.44	32.2	35.8	4.28	138	153	+15	±24	
35	2.57	34.1	36.6	4.36	149	159	+10	±25	
26	2.67	39.5	37.2	5.92	234	220	-14	±27	
20	2.80	43.6	37.9	5.92	258	224	-34	±27	-7
36	2.87	41.5	38.3	9.35	388	358	-30	±31	
49	3.11	40.9	39.7	4.88	199	194	-5	±26	
47	3.19	39.7	40.2	5.74	228	231	+3	±27	
47	3.19	41.0	40.2	5.68	233	228	-5	±27	
47	3.20	39.0	40.3	5.84	228	235	+7	±27	
49	3.30	43.4	40.9	4.98	216	204	-12	±26	
49	3.30	39.6	40.9	5.10	202	208	+6	±26	
47	3.34	40.8	41.1	5.64	230	232	+2	±27	
49	3.46	39.8	41.8	5.18	206	217	+11	±27	
32	3.49	42.7	42.0	7.61	325	320	-5	±30	
49	3.52	47.6	42.2	5.15	245	217	-28	±27	-1
31	3.60	43.4	42.6	6.38	277	272	-5	±28	
49	3.61	45.7	42.7	6.10	279	260	-19	±28	
31	3.62	43.7	42.8	6.58	288	281	-7	±28	
47	3.67	36.3	43.1	6.71	244	289	+45	±29	+16
31	3.67	37.5	43.1	6.33	237	273	+36	±28	+8
23	3.70	41.3	43.2	6.27	259	271	+12	±28	
49	3.70	42.1	43.2	5.25	221	227	+6	±27	
38	3.73	44.6	43.4	7.15	319	310	-9	±29	
32	3.73	46.3	43.4	8.09	375	351	-24	±31	
32	3.80	42.6	43.8	7.44	317	326	+9	±30	
52	3.82	43.0	43.9	6.45	277	283	+6	±29	
23	3.83	46.4	44.0	6.66	309	293	-16	±29	
33	3.94	43.3	44.6	6.50	281	290	+9	±29	
42	3.98	45.9	44.9	6.99	321	314	-7	±29	
48	4.01	44.9	45.1	6.82	306	307	+1	±29	
22	4.03	45.0	45.2	6.71	302	303	+1	±29	
38	4.04	43.8	45.2	6.98	306	316	+10	±30	
23	4.04	45.7	45.2	7.12	325	322	-3	±30	
10	4.05	47.6	45.3	6.49	309	294	-15	±29	
10	4.10	49.1	45.6	6.38	313	291	-22	±29	

TABLE I (continued)

Case number	Albumin	Specific pressure observed	Specific pressure calculated	Total protein	Osmotic pressure observed	Osmotic pressure calculated	Variation of calculated from observed pressure	Maximum experimental error	Unexplained variations
	grams per 100 cc.	mm H ₂ O	mm H ₂ O	grams per 100 cc	mm H ₂ O	mm H ₂ O	mm. H ₂ O	mm H ₂ O	mm H ₂ O
7	4 11	44 2	45 6	7 95	352	363	+11	±31	
12	4 13	47 1	45 8	6 33	298	290	- 8	±29	
16	4 14	44 1	45 8	7 01	309	321	+12	±30	
33	4 16	44 3	45 9	6 77	300	311	+11	±29	
23	4 19	44 2	46 1	6 92	306	319	+13	±30	
4	4 22	46 4	46 3	7 06	328	327	- 1	±30	
31	4 22	49 2	46 3	7 54	371	349	-22	±30	
17	4 23	45 8	46 4	7 08	324	328	+ 4	±30	
17	4 25	49 4	46 5	6 82	337	317	-20	±30	
17	4 27	47 7	46 6	6 67	318	311	- 7	±29	
28	4 27	48 6	46 6	5 99	291	279	-12	±29	
24	4 30	49 1	46 8	6 49	319	304	-15	±29	
3	4 33	47 2	46 9	6 65	314	312	- 2	±29	
50	4 33	45 5	46 9	8 18	372	384	+12	±31	
3	4 36	48 7	47 1	6 47	315	305	-10	±29	
29	4 37	48 0	47 2	7 06	339	333	- 6	±30	
6	4 38	45 4	47 2	7 11	323	336	+13	±30	
25	4 39	45 0	47 3	7 25	326	343	+17	±30	
4	4 39	48 0	47 3	7 11	341	336	- 5	±30	
17	4 40	47 7	47 4	6 64	316	314	- 2	±30	
9	4 41	47 3	47 4	7 06	334	335	+ 1	±30	
16	4 42	46 4	47 5	6 70	311	318	+ 7	±30	
8	4 42	47 2	47 5	6 80	321	323	+ 2	±30	
2	4 43	42 8	47 5	6 48	277	308	+31	±29	
30	4 44	47 0	47 6	7 10	334	338	+ 4	±29	
15	4 46	49 8	47 7	6 26	312	299	-13	±29	
44	4 46	48 4	47 7	6 92	335	330	- 5	±30	
43	4 48	51 2	47 8	6 64	340	318	-22	±30	
9	4 49	48 9	47 9	6 99	342	335	- 7	±30	
29	4 50	45 7	48 0	7 10	325	340	+15	±30	
8	4 50	51 0	48 0	7 12	363	341	-22	±30	
24	4 52	46 3	48 1	6 78	314	326	+12	±30	
29	4 52	44 9	48 1	7 07	317	340	+23	±30	
5	4 52	49 8	48 1	6 33	315	304	-11	±29	
17	4 53	47 2	48 1	7 44	351	358	+ 7	±31	
3	4 54	48 3	48 2	6 63	320	319	- 1	±30	
51	4 55	52 8	48 2	6 35	335	306	-29	±29	
21	4 57	49 2	48 4	6 69	329	324	- 5	±30	
8	4 59	46 8	48 5	6 72	314	326	+12	±30	
30	4 59	49 6	48 5	6 86	340	333	- 7	±30	
30	4 60	48 8	48 5	7 27	355	353	- 2	±31	
30	4 62	44 8	48 7	7 12	319	346	+27	±30	
39	4 62	48 6	48 7	6 85	333	333	0	±30	
8	4 62	48 2	48 7	6 91	333	336	+ 3	±30	
15	4 63	51 2	48 7	6 53	334	318	-16	±30	
41	4 64	51 5	48 8	6 87	354	335	-19	±30	

+ 2

TABLE I (continued)

Case number	Albumin	Specific pressure observed	Specific pressure calculated	Total protein	Osmotic pressure observed	Osmotic pressure calculated	Variation of calculated from observed pressure	Maximum experimental error	Unexplained variations
	grams per 100 cc	mm H ₂ O	mm H ₂ O	grams per 100 cc	mm H ₂ O	mm H ₂ O	mm H ₂ O	mm H ₂ O	mm H ₂ O
6	4.68	47.6	49.0	6.74	321	330	+9	±30	
24	4.68	51.6	49.0	7.02	362	344	-18	±30	
18	4.69	50.5	49.1	7.92	400	389	-11	±31	
24	4.72	48.9	49.2	7.12	348	351	+3	±31	
34	4.72	50.5	49.2	6.87	347	338	-9	±30	
46	4.77	50.3	49.5	7.51	378	372	-6	±31	
11	4.77	50.0	49.5	7.13	357	353	-4	±31	
24	4.78	53.3	49.6	6.64	354	329	-25	±30	
13	4.78	50.4	49.6	6.91	348	343	-5	±30	
19	4.78	49.1	49.6	7.03	345	349	+4	±31	
53	4.79	51.9	49.7	7.14	371	355	-16	±31	
27	4.81	51.4	49.8	7.02	361	349	-12	±31	
12	4.84	51.4	50.0	7.50	385	375	-10	±31	
15	4.90	49.3	50.3	6.78	334	341	+7	±30	
14	4.91	48.5	50.4	7.58	368	382	+14	±31	
24	4.91	49.2	50.4	6.95	342	350	+8	±31	
24	4.92	49.1	50.4	7.07	348	357	+9	±31	
8	4.92	55.7	50.4	7.95	443	401	-42	±32	-10
45	5.01	51.2	51.0	6.86	351	350	-1	±31	
12	5.02	49.0	51.0	7.70	377	393	+16	±32	
40	5.03	51.6	51.1	7.03	363	359	-4	±31	
1	5.06	52.3	51.3	6.86	359	352	-7	±31	
21	5.16	53.0	51.8	7.55	400	391	-9	±32	
29	5.18	53.6	52.0	8.26	443	429	-14	±32	
4	5.22	49.8	52.2	7.72	384	403	+19	±32	
34	5.32	54.0	52.8	7.96	430	420	-10	±32	
19	5.36	57.9	53.0	7.72	447	409	-38	±32	
13	5.44	52.2	53.5	8.07	421	432	+11	±33	
3	5.45	55.2	53.6	7.92	437	424	-13	±32	
53	5.48	54.2	53.7	7.82	424	420	-4	±32	
17	5.49	54.6	53.8	8.54	467	459	-8	±33	
24	5.55	52.1	54.1	8.11	423	439	+16	±33	
19	5.60	54.8	54.4	8.30	455	452	-3	±33	
21	5.93	56.2	56.4	8.66	487	488	+1	±34	
13	5.94	49.6	56.4	8.70	432	491	+59	±34	+25
53	6.08	57.4	57.3	9.05	519	518	-1	±34	
15	6.14	56.9	57.6	8.68	494	500	+6	±34	
34	6.62	59.2	60.5	9.64	571	583	+12	±36	

seen to be quite definitely linear, this fact being indicated clearly by the general appearance of the surface produced by the tips of the wires, which lay, in general, in a plane which was parallel to the G-axis and inclined to the A-axis. These characteristics of the relationships are brought out in Figure 1, which shows the total lack of correlation between S and G and in Figure 2, which shows the high degree of correlation

TABLE II
Tabulation of errors of calculation and experimental errors

	Albumin	Total protein	Specific osmotic pressure	Total osmotic pressure
Variations of calculated from observed values				
Absolute variations			mm H ₂ O	mm H ₂ O
Standard error			± 2.23	
Average variation			± 1.74	± 11.56
Maximum variations			+ 6.8	+ 59.0
			- 5.7	- 42.0
Per cent variations			per cent	per cent
Standard deviation			± 5.05	± 5.05
Average per cent variation			± 3.87	± 3.89
Maximum per cent variations			+ 18.7	+ 18.4
			- 13.1	- 13.2
Experimental errors				
	grams per 100 cc	grams per 100 cc		mm H ₂ O
Differences in values of duplicate determinations				
Average difference	0.08	0.09		5.1
Maximum differences	0.39	0.66		19.0

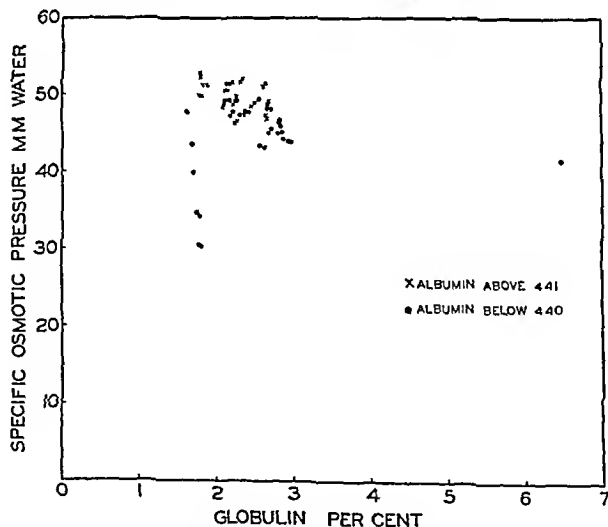


FIG 1 Showing complete lack of correlation between specific osmotic pressure and globulin concentration. Higher values of S correspond to high values of A, only and lower values of S correspond to lower values of A, only regardless of values of G.

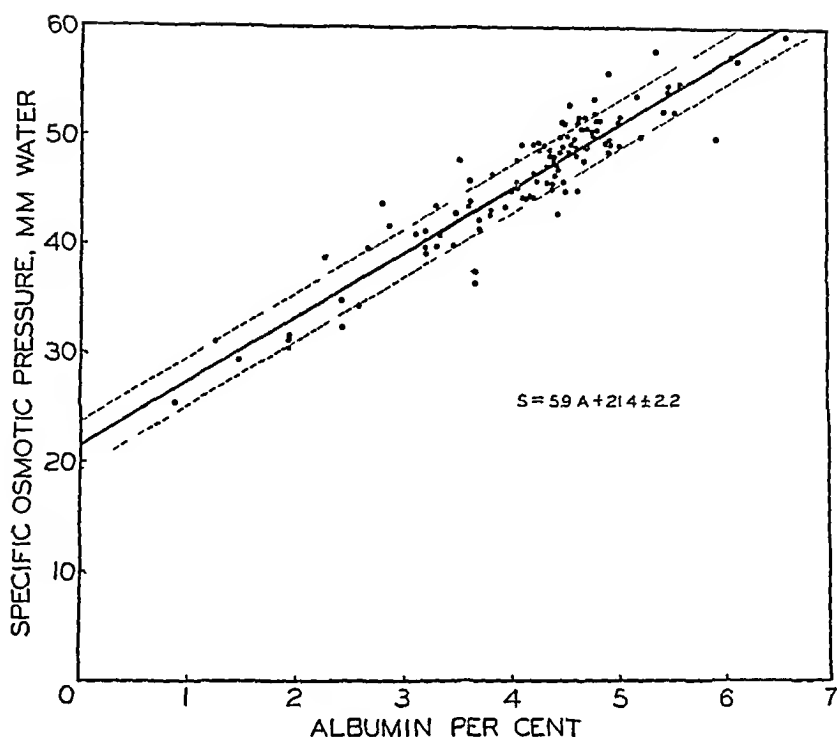


FIG. 2 Showing the linear relation of specific osmotic pressure to albumin concentration, as a straight line, fitted by the method of least squares. Correlation coefficient of S and A is 0.93. Standard error of calculation, indicated by dotted lines, is ± 2.2 mm.

between S and A. The Pearson coefficient of correlation between S and A was found to be 0.93, which indicates a high degree of relationship between the variables. The heavy line drawn through the points on the S-A graph is a straight line fitted to the data by the method of least squares. The equation of this line, $S = 5.9A + 21.4$ is corrected by means of the dotted lines on either side for the "standard error" of the calculated as compared to the observed values. The standard error being ± 2.2 mm of water, it may be prophesied on grounds of probability that, in any other similar series of measurements, the observed values of S will, in 68 per cent of the cases, fall within the range of values calculated from the formula $S = 5.9A + 21.4 \pm 2.2$. Similarly, if the correction be doubled (twice the standard error being ± 4.4) the range will include 95 per cent of observed values, while if the correction be trebled 99.7 per cent of cases should be included. Actually, in our series the percentage of cases included by the corrections corresponding respectively to the standard error, to twice the standard error, and to three times the standard error, are 72.6, 93, and 98.5. The frequency distribution of the errors is therefore quite according to the "normal distribution." The average

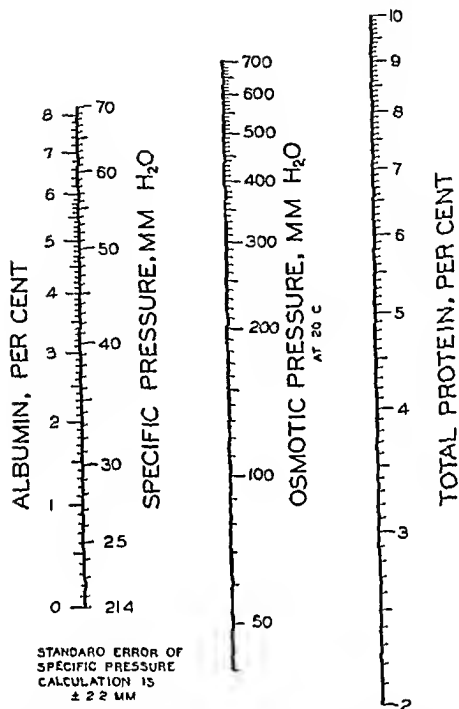


FIG 3 Nomogram A straight line drawn through the proper points on the albumin and total protein scales will intersect the middle scale at the value of the total osmotic pressure calculated from the formula $P = C(21.4 + 5.9 A)$

error of calculation of S was ± 1.74 mm and the maximum errors encountered were $+6.8$ and -5.7 mm

The complete independence of the specific osmotic pressure from any effect of varying concentrations of globulin is exemplified by the findings on a sample of serum from the patient (Case Number 36) who suffered from Banti's disease. The albumin concentration was 2.87 per cent and the globulin concentration was 6.48 per cent, giving an A/G ratio of 0.44. The observed specific osmotic pressure was 41.5 mm of water, while the value of S calculated from the albumin concentration is 38.3. On the assumption that the globulin fraction of serum exerts, universally, a lower osmotic pressure, gram for gram, than the albumin fraction, one

should expect the value of S of a serum having such an unusually high globulin content to be lower than that calculated by our formula. The fact that it was actually *higher*, by nearly 8 per cent, indicates clearly the unreliability of the A/G ratio as an index of the specific osmotic pressure, and confirms the idea that it is the albumin concentration which determines the specific osmotic properties of the serum.

The total osmotic pressure, P , is calculated from the formula $P = C(5.9A + 21.4)$. The nomogram, reproduced in Figure 3, may be used to facilitate the calculations. The absolute error of the calculation of P is equal to the error of S multiplied by the concentration of total protein, C . The average error for our series was ± 11.6 mm, the maximum errors being $+59$ and -42 mm. It is the percentage error of calculation of the total osmotic pressure, however, which will be most significant in practice. The percentage error will, in general, be the same for S as for P , and for our series the average per cent error is slightly less than ± 3.9 , the maximum errors being $+18.7$ and -13.2 per cent. The standard deviation of the per cent errors about their mean (which is -0.1) is ± 5.05 , which signifies that 68 out of every 100 values of S or P , calculated by the given formulae, will correspond to the observed values within approximately ± 5 per cent, and similarly 99.7 out of every 100 calculated values should check the observed values within ± 15 per cent. Actually, errors lying within the ranges ± 5 per cent and ± 15 per cent occurred in 77 and 99 per cent of cases, respectively. The per cent errors of calculation may therefore be considered as being "normally" distributed.

Sources of error

The term "observed value," as used in the last paragraph, refers, in each case, to the average of the two values obtained from duplicate osmotic pressure determinations. However, in 4 instances, due to a scarcity of serum, only a single determination was run, but in only 1 of these cases was the variation between the observed and calculated values as great as 5 per cent. The greatest variation between the two values obtained from duplicate determinations was 19 mm. The average variation was 5.1 mm and 95 per cent of the cases showed variations which were not greater than 12 mm. In general there was no relation between the closeness of the check of the duplicate osmotic pressure determinations and the degree of variation of the observed mean pressure from the pressure calculated from the protein analyses.

The question naturally arises as to what extent the variations of the calculated from the observed pressures may be accounted for on the basis of experimental errors and to what extent they must be considered as due to unknown factors. We should like to be able to decide to what extent the osmotic pressure of colloids of serum is due to the protein concentrations as we have measured them and to what extent it is due

to other factors that were not measured during these investigations. The average variation of calculated from observed values was ± 11.5 mm while the average variation of duplicate osmotic pressure determinations was only 5.1 mm, or ± 2.55 mm when related to the means of the pairs of values. Unfortunately the variation of duplicate determinations is not always a measure of the error of a method. "Good checks," while reassuring, do not exclude the possibility that systematic errors may have affected both values to the same extent and in the same direction. Allowing for the possibility that systematic errors were present of a type which could affect our pairs of observations now in one direction, now in another, at random, we should be fairly safe in assuming, arbitrarily, that the "maximum expected error" will not be greater than ± 12 mm, which is twice the variation from the mean of duplicate determinations in 95 per cent of our cases. We can neglect the possibility that systematic errors may operate in one direction for the whole series of cases for they could not be responsible for variations of observed values from values calculated by a formula based on the same series of observations.

The error of the chemical analysis of the albumin and total protein concentrations will contribute its share to the total error of the calculated value of the osmotic pressure. In 93 per cent of the cases the variations between duplicate determinations of these quantities were not greater than 0.2 gram per 100 cc. Assuming that the maximum expected error is ± 0.2 gram per 100 cc for albumin and also for total protein, the maximum, additive error of both analyses will be responsible for a possible error of from ± 10 to ± 20 mm in the calculation of the osmotic pressure. By the method of differentials, where the pressure is calculated by the formula $P = C(21.4 + 5.9A)$, the total error is the total differential, dP . Now, $dP = 21.4dC + 5.9AdC + 5.9CdA$. Since dA and dC , the errors of the analyses, are both considered as ± 0.2 , we have $dP = \pm [1.2(A + C) + 4.3]$. The maximum error of analysis was calculated in this way for each case, and the maximum experimental error found by adding the maximum error of the osmotic pressure determination, which is considered to be ± 12 mm. The figures are given in the last column of Table I. Even with so great an allowance for errors of measurement, there were 6 instances in which the errors of calculation could not be accounted for. It is therefore necessary to assume that other factors, which were not measured, have a determining influence upon the osmotic pressure exerted by a given sample of serum. We may conclude that, whereas in a *series* of cases the errors due to the unknown factor or factors will not seriously affect the reliability of the calculation of the osmotic pressure from the albumin and total protein concentration, in any *single instance* the calculated value may possibly be in error by an amount which is much greater than the sum of the

experimental errors of the methods employed. The unknown factor does not seem to be referable to individual ("personal") peculiarities of the blood, for in no instance did a large error occur more than once in the series of determinations carried out on blood samples from individual subjects. It may be assumed that the method of calculation will give reliable results when applied to a series of determinations on the same individual as well as when applied to single determinations on a series of several individuals. As a matter of fact, the greatest error so far encountered, 19 per cent, is not so great as to discourage the employment of the method, for most clinical purposes, for single determinations on single individuals. In this respect the method cannot be considered to be less accurate than the usual determination of the basal metabolic rate, for which an error of ± 15 per cent is ordinarily allowed.

The significance of the relation between the specific osmotic pressure and the total protein and albumin concentrations. It has been pointed out by Marrack and Hewitt (7) that the "Donnan pressure" due to the excess of diffusible ions in a protein solution over that in the outside solution will be proportional to the square of the protein concentration and must be added to the osmotic pressure of the protein alone in calculating the total osmotic pressure of an ideal protein solution. Theoretically, therefore, $P = aC + bC^2$, where a is the constant of proportionality relating the pressure due to the protein alone to the concentration, and where b is a constant relating to the Donnan pressure at constant pH. The equation, of course, will be expected to hold for a series of blood sera only if they contain the same proportions of the two protein fractions, albumin and globulin—that is to say, when A/G remains constant. But when A/G is constant, C is directly proportional to A, so that our equation for the total osmotic pressure, $P = 21.4C + 5.9AC$, may be written $P = 21.4C + 5.9bC^2$, where b is the constant of proportionality between C and A for a constant value of A/G. This is the theoretical form of equation for the Donnan effect. Now $S = P/C$, so that the theoretical formula reduces to $S = a + bC$. For constant values of A/G our equation may be reduced to the identical form, $S = 21.4 + 5.9bC$.

Interesting though these comparisons of our empirical equations with the theoretical Donnan equations may be, however, they do not completely explain the peculiar relations which we have found to exist. It is difficult to understand, on any theoretical basis which is not entirely speculative, why the specific pressure should be so completely independent of the globulin concentration and hence of the A/G ratio. The relation would seem to imply that the specific pressure of globulin in any serum is the same as that of the albumin with which it is associated, and that it has a value which is directly determined by the concentration of the albumin. On the other hand, globulin does not appear to have any effect on the specific pressure of albumin. It should be emphasized that

the term "specific pressure" refers not to the total osmotic pressure, but to the average pressure per gram per cent of total protein. The globulin content of a given serum appears to contribute gram for gram exactly as much to the total pressure as does the albumin. Consequently our data offer no justification for the assumption that albumin (in serum) has an effective molecular weight which is much less than that of globulin. The actual molecular weights of the two substances may, of course, be widely different but if they are, the fact is not reflected in our data and we must assume that other factors enter in to mask the effects of a difference in molecular weight. Whatever the explanation of this peculiar situation may be, the existence of the relation indicates the need for an experimental study of the osmotic properties of both the separate and the mixed solutions of purified serum albumin and serum globulin.

The finding that the albumin concentration determines the specific osmotic pressure, not only of the albumin itself but also of the accompanying globulin (regardless of the concentration of the globulin) is of some significance in relation to the clinical correlation of edema with low serum albumin concentrations for it becomes clear that a progressive lowering of the albumin content of a patient's serum entails a simultaneous lowering of the specific osmotic properties of the accompanying globulin fractions, even if the globulin concentration is not lowered.

The significance of the A/G ratio as an index of the osmotic properties of serum depends, apparently, on the *clinical* fact that changes in this ratio are *most often* due to changes in the albumin fraction—the globulin changing relatively less, in most instances. However, in cases where the globulin undergoes marked variations from the normal value the A/G ratio becomes totally unreliable as an index. In our *series as a whole* there is a fair degree of correlation between the specific pressure and the A/G ratio, but this must be attributed solely to the particular selection of cases. In individual cases the correlation was often very poor indeed.

The fact that the specific pressure increases with increasing total protein concentration (except when this increase is due to an increase in G alone) is in accord with the findings of many other investigators for serum, (8, 9, 10), for serum albumin (11), for other pure protein solutions (12, 13) and for all types of concentrated solutions of crystalloids so far studied, of which the classical example is sucrose (14). Consequently it would appear that the contention of Govaerts that the total protein concentration is without influence on the specific osmotic pressure is without justification except from his own data.

The apparent unreliability of the formulae of Govaerts and of von Farkas
In general the values obtained by Govaerts for the osmotic pressure of serum tend to be higher than those obtained by others. Further evidence that the values upon which Govaerts' formula is based are unreliable is given by the fact that Verney (8), employing cellophane membranes

furnished by Govaerts, obtained values which were lower in relation to the protein concentrations than those of Govaerts, but of the same order of magnitude as those of most other workers

We have used the formula of Govaerts, $P = 75.4A + 19.5G$, to calculate the osmotic pressures of our sera and find that these calculated values are, on the average, 18.4 per cent higher than the values which we observed, with a range of variation of -3 to $+59$ per cent. Since the formula of Govaerts fits his own data very well, we may conclude from the degree of variability found by the above-mentioned calculations that his observed pressures, mostly higher than ours, are not higher in any definite proportion but in a totally irregular manner. If our determinations are correct, as we believe them to be, it follows that Govaerts' formula cannot be relied upon to give calculated values which are even *relatively* accurate. The same criticism may be applied to the formula of von Farkas, $P = 68A + 25.1G$, for the application of this formula to our data provides values which are, on the average, 13.4 per cent higher than the values observed by us, the range of variation being -8 to $+56$ per cent.

SUMMARY

1 The osmotic pressure of colloids and the total protein, albumin and globulin concentrations of 128 samples of human serum were determined. The samples were obtained from 53 individuals.

2 Analysis of the data, which cover a relatively wide range of values, indicates that the relation between the total protein and albumin concentrations and the osmotic pressure is sufficiently regular and definite to allow the use of an empirical formula, derived from the data, for the calculation of the osmotic pressure. The formula is $P = C(21.4 + 5.9A)$, where P is the osmotic pressure in millimeters of water, C is the total protein concentration and A is the albumin concentration, in grams per 100 cc. The standard error of calculation is ± 5 per cent.

3 A nomogram has been constructed to facilitate calculations.

4 The specific osmotic pressure of serum appears to be a linear function of the albumin concentration. Variations in the globulin concentration over a wide range produce no effect on the specific pressure at constant values of A . Consequently the A/G ratio is a poor index of the osmotic properties of serum. The specific pressure increases for increasing values of the total protein concentration, but only in so far as these increases are not due to increases in the globulin. The theoretical significance of these relations is discussed.

5 The formulae of Govaerts and of von Farkas, which have been used to calculate the osmotic pressure, appear to be totally unreliable. The reasons for this conclusion are discussed.

BIBLIOGRAPHY

- 1 Govaerts, M P, Bull Acad roy de méd de Belgique, 1927, xiii, 356
Influence de la teneur du sérum en albumines et en globulines sur la
pression osmotique des protéines et sur la formation des oedèmes
- 2 v Farkas G, Ztschr f d ges exper Med, 1927, lvi, 666 Studien über
den kolloidosmotischen Druck des Serums
- 3 Burk, N F and Greenberg D M, J Biol Chem, 1930, lxxxvii, 197
The physical chemistry of the proteins in non aqueous and mixed
solvents I The state of aggregation of certain proteins in urea water
solutions
- 4 Wells, H S, Am J Physiol, 1932, ci, 409 Some criteria of accuracy
for the measurement of the osmotic pressure of colloids in biological
fluids
- 5 Wells H S, J Tenn Acad Sci, 1933 viii, 102 A modified Krogh
Osmometer for the determination of the osmotic pressure of colloids
in biological fluids (with directions for its use)
- 6 Howe, P E, J Biol Chem 1921 xlix, 109 The determination of
proteins in blood—a micro method
- 7 Marrack J and Hewitt, L F, Biochem J, 1927, xxi, 1129 The effect
of hydrogen ion concentration and protein concentration on the osmotic
pressure of serum proteins
- 8 Verney, E B, J Physiol, 1926, lvi, 319 The osmotic pressure of the
proteins of human serum and plasma
- 9 Krogh, A and Nakazawa, F, Biochem Ztschr, 1927, clxxxviii, 241
Beiträge zur Messung des kolloid osmotischen Druckes in biologischen
Flüssigkeiten
- 10 Fishberg, E H J Biol Chem, 1929 lxxxi, 205 The relations of the
serum proteins and lipids to the osmotic pressure
- 11 Burk, N F, J Biol Chem (Proc.) 1931, xcii, xl The molecular weight
of serum albumin estimated by osmotic pressure measurements
- 12 Kunitz, M, J Gen Physiol 1926-27, x, 811 Hydration of gelatin in
solution
- 13 Adair, G S, Proc Roy Soc London Series A, 1925 cix, 292 The
osmotic pressure of hemoglobin in the absence of salts
- 14 Porter A W, Tr Faraday Soc, 1917, xii, 123 The kinetic theory of
osmotic pressure

HERPETIC PHARYNGITIS AND STOMATITIS A REPORT OF THREE CASES

By PERRIN H LONG

*(From the Medical Clinic of the Johns Hopkins Hospital and the Department of Medicine,
Johns Hopkins University Baltimore)*

(Received for publication July 31, 1933)

In recent years great interest has been manifested in the virus of herpes simplex. It has been stimulated by an increasing curiosity regarding filterable viruses in general, and by the possibility that herpes virus may have a causal relationship to epidemic encephalitis. However, despite the enthusiasm for the study of the properties of this filterable agent, little attention has been paid to the natural manifestations of the disease herpes simplex. This may be due to the ubiquity of herpes simplex or it may be possible that the essential herpetic character of many affections is not recognized.

The recognition of herpes as a clinical entity is probably of great antiquity, the word itself being derived from the Greek *ἑρπης* meaning literally "a creeping." Aetaeus the Cappadocian, in discussing affections of the tonsils, describes small superficial ulcers to which he gave the name *aptha*. It is not unlikely that these lesions were herpetic in nature. In 1398, John Trevisa in his fragmentary translation of Bartholomaeus Anglicus' manuscript, "On the Property of Things," makes mention of "this euyl is callyd Herpes" and "suche a scabbe highte Herpes Cingula." This constitutes the first reference to herpes in the English language, and it is plain from this citation that herpes was recognized in the Middle Ages. During the succeeding centuries one finds a constant increase in the number of references to herpetic infections, thereby demonstrating the interest attached to the disease.

However, it was not until the beginning of the nineteenth century that a clear differentiation was established between the clinical course of herpes simplex and herpes zoster. Following this distinction one finds an increased interest taken by clinicians in the delineation of the various simple herpetic affections. The general manifestations of the disease as well as the local, occupied their attention and in the middle of the century, several splendid clinical reports describing the disease are to be found.

Gubler (1) in 1858 gives an admirable account of herpetic infections of the throat which we will quote. "A la suite d'un refroidissement, un sujet est pris de malaise, de courbature, puis d'une fièvre quelquefois

assez intense, ainsi que d'un mal de gorge les deux amygdales sont tuméfiées, rouges et ne tardent pas à présenter des surfaces circulaires ou irrégulièrement configurées, semblables à des ulcérations superficielles couvertes d'une exsudation plastique grisâtre ou jaunâtre, souvent cette dernière lésion est unilatérale. En même temps il apparaît sur les lèvres une éruption d'herpès ordinairement groupée en grand partie vers l'une des commissures. Tel est aussi le type consacré par la description classique. Eh bien, dans mon opinion, ces ulcérations tonsillaires doivent être considérées comme de l'herpès labialis sur une surface muqueuse."

Trousseau (2) devoted an entire clinic to the description of herpetic pharyngitis. His description of the individual lesions is classical. "On voit en effet des taches blanches, entourées d'une aréole inflammatoire assez étendue, et dont le volume varie depuis le gros grain de millet jus qu'à celui d'un pois. Ces taches laissent à leur place des ulcérations superficielles dont les bords peuvent néanmoins être saillants en raison du gonflement oedémateux des tissus environnants envahis par l'inflammation."

In 1881 James Wilson in his book on "The Continued Fevers" describes simple continued fever as "a fever not due to any specific cause, usually of short duration, lacking the distinguishing characteristics of other fevers, and rarely fatal in temperate climates. The eruption of herpes upon the lips and nose is so common at the close of simple continued fever that this disease by some persons has been called Herpetic Fever."

Two years later Savage (3) reported an epidemic of herpetic fever which occurred in an English boys' school. The clinical picture of the disease in this epidemic was characterized by a slow onset accompanied by headache, lassitude, chilliness, fever, irritability, malaise and vomiting. After twenty-four hours, patches of herpetic lesions appeared about the lips or lobes of the ears. In two individuals vesicles appeared on the lower extremities. With the appearance of the vesicles the patients complained of sore throats. Frequently herpetic lesions were present in the throat. By the fourth day the temperature was normal and the patients were convalescent. Thirty-nine boys were affected during a two-week period.

In recent years, despite Trousseau's remark that "Au jour d'hui qu'on est suffisamment averti, il est peu de médecins qui n'aient en l'occasion d'en observer des exemples," very little can be found in medical literature regarding the clinical course of localized and generalized herpetic infections of the simplex type. In 1932 Youmans (4) described an individual ill from herpetic fever with stomatitis. He was successful in isolating the virus of herpes simplex from the mouth lesions in this patient, thus for the first time demonstrating the essential etiological character of the disease.

In this report we wish to discuss three cases of herpetic infections in the mouth, two of which were accompanied by fever. From two of the individuals the virus of herpes simplex was recovered.

CASE HISTORIES

Miss C. K. J. H. H. 40611, a student aged 23 entered the Medical Clinic on December 9, 1931, complaining of a "sore throat and swollen tender gums" of three days duration. Her past and family history was unimportant. Three days before coming to the clinic the patient had spent the week end in New York and while riding to Baltimore on the train, she noticed that her throat was sore. Upon the following day she experienced "chills and fever." The soreness in the throat increased and the gums became tender and swollen. Headache appeared and some generalized aching was present. On December 8th the symptoms were more marked and the throat affection was so severe that swallowing became very difficult. She was admitted to the Isolation Ward on the following morning.

Physical examination. The patient was undernourished and seemed quite uncomfortable. The temperature was 101.6° F., the pulse 112, and the respirations were 20. The skin was warm and moist; the ears, eyes and nose were normal. The lips were dry and cracked and just to the right of the midline was a small, crusted, superficial ulcerated lesion. The gums were tender, swollen and spongy and there were numerous small superficial lesions capped with white membranes on the tooth gum margins. These bled freely when slightly traumatized. Near the tip of the tongue were several small vesicles as well as upon the hard palate. The tonsils had been cleanly removed, but the soft palate, uvula, pillars and posterior pharyngeal wall were intensely inflamed and moderately swollen. The lymphoid follicles of the posterior pharyngeal wall were markedly hypertrophied and there were numerous small superficial vesicular lesions over the posterior pharyngeal wall. The regional lymph nodes were enlarged and slightly tender as were also the axillary glands. The remainder of the physical examination was essentially normal.

Laboratory data. Urine normal. Red blood cells 4.4 million. Hemoglobin 90 per cent. White blood cells 4,560. Differential count—Polymorphonuclears 72.5, lymphocytes 12.5, monocytes 15. Wassermann reaction negative. Throat cultures (7), staphylococcus aureus. Throat smears—no Vincent's organisms. Blood culture negative.

Impression. It was thought on admission to the Ward that the patient was suffering from an acute septic sore throat and an aphthous stomatitis. The possibility of a Vincent's infection was considered, but at no time were Vincent's organisms demonstrated.

Course. The temperature remained elevated on the day after admission and the total white blood cells numbered 6,000. The patient complained of a severe sore throat. The mouth lesions were unchanged. On December 11th the temperature was lower and the white blood cells totaled 5,950. The white membranes capping the vesicles were beginning to slough off leaving clean superficial ulcers. On December 12th the temperature fell to normal and there was a marked symptomatic improvement. White blood cells 3,750. Treatment of the ulcers by topical application of 2 per cent gentian violet was instituted. During the next few days the patient improved and by December 18th her mouth and throat were free from ulcerated areas. On December 22nd she was discharged well, and as far as we know she has never had a recurrence of this affection.

Mr M T, J H H 46235, a white, night club saxophone player, aged 25 years, entered the Medical Clinic on November 4th, 1932, complaining of a "sore mouth" of six days duration. His past and family history was unimportant. Five days before coming to the hospital the patient felt "feverish" and thought that his tongue and gums were dry and swollen. A moderately severe headache was present. By evening he developed definite soreness of the throat, tongue and gums. On the following day he felt "grippy" and suffered from generalized aching and malaise. By the next day his throat was so sore and he felt so seedy that he took to his bed. Profuse sweats occurred, his neck became swollen and tender and his headaches more severe. He suffered from insomnia and on the night before entry he was disoriented. All of his symptoms increased in intensity and he came to the hospital.

Physical examination The patient was well developed and well nourished, but apparently was very uncomfortable. The temperature was 100° F, the pulse 96, and the respirations 22. The skin was hot and dry, the ears, eyes and nose were normal. The lips were dry, fissured and scaly. Several small superficial crusted ulcers were present on the outer dry surface of the lips. The inner surface of the lips, the buccal mucous membranes, and the gums were dull red in color. Many deflated vesicles capped with dead white skin were seen on these surfaces. The gums bled when slightly traumatized. The tongue was beefy red and was covered with numerous vesicles. Many similar lesions were seen on the hard palate. The soft palate, uvula, pillars, and posterior pharyngeal wall were moderately inflamed and slightly swollen. On the soft palate, uvula and right posterior pillar there were small vesicles surrounded by a small area of intense hyperemia and capped with a dull chalk-white tense membrane. These collapsed completely when the vesicular fluid was released. The regional and anterior cervical lymph nodes were enlarged and tender. Otherwise the physical examination was normal.

Laboratory data Urine normal. Red blood cells 4.6 million. Hemoglobin 106. White blood cells 6,880. Differential count—polymorphonuclears 62, eosinophiles 1, basophils 1, lymphocytes 34, monocytes 2. Wassermann reaction negative. Throat smears—no Vincent's organisms. Throat culture—staphylococcus aureus.

Impression Because of the presence of typical herpetic lesions upon the lips and the vesicular character of the eruptions in the mouth, it was thought that this patient was suffering from herpetic stomatitis and pharyngitis.

Course On the day following the patient's admission to the hospital, his temperature was normal, but there was no change in the condition of the mouth. The mouth lesions were treated with topical applications of gentian violet. On November 8th the white cells numbered 7,700. The lesions were slowly resolving, but enough discomfort persisted to prevent the taking of solid food. By November 11th the lesions had practically disappeared and the patient was discharged. There has been no recurrence of the herpes up to the present time.

Miss C W, J H H 46136, a student nurse, aged 22, entered the Medical Clinic on January 2nd, 1933, complaining of a "sore throat" of two days duration. With the onset of this affection she suffered from "chills and fever," headache and anorexia. On the following day she felt weak and experienced generalized aching. Profuse sweats occurred during the night. On the day of admission malaise was present and the glands in her neck were enlarged and tender.

Physical examination The patient was well nourished and seemed com-

fortable The temperature, pulse and respirations were normal A faint, generalized erythema was present The ears, eyes and nose were normal, as were also the lips gums, buccal mucous membranes and tongue A discrete vesicular eruption was present over the left anterior pillar and over a right tonsillar tag The vesicles ranged in size from two to four millimeters and were surrounded by small areas of very hyperemic mucous membrane The entire throat was mildly erythematous and the lymphoid follicles on the posterior pharyngeal wall were hypertrophied The regional lymphnodes were moderately enlarged and tender Otherwise the physical examination was normal

Laboratory data Urine normal Red blood cells 4.9 million Hemoglobin 100 White blood cells 7,050 Differential count—polymorphonuclears 65, eosinophiles 1 basophils 1, lymphocytes 29, monocytes 4 Wassermann reaction negative Throat culture—staphylococcus albus and aureus

In presson Herpetic pharyngitis

Course The temperature, pulse and respirations remained normal throughout the patient's stay in the hospital By January 6th the throat had improved and on the following day the patient was discharged from the hospital No local treatment was used in this instance There has not been any recurrence of the affection to date

Experimental studies

Miss C K No studies were made because the nature of the throat affection was not suspected until after the vesicles had disappeared

Mr M T Upon November 4th, the cap of a tense vesicle was removed with biting forceps and this material was rubbed into the scarified left corneae of two rabbits Within twenty four hours a localized injection of the sclera was observed and after a short time one could see minute vesicles along the lines of scarification with the aid of a hand lens By November 7th a full blown, steamy keratitis was manifest in both animals At this point, rabbit Number 1 was killed, the left cornea was removed and then ground in a sterile mortar with a small amount of sterile physiological saline solution The left scarified corneae of rabbits Numbers 3 and 4 were inoculated with the ground material

On the 12th of November rabbit Number 2 was hypersensitive and circled definitely to the left Three days later this animal was extremely hypersensitive, circled to the left, showed marked salivation and retention of urine, ran blindly into objects and had an accelerated respiratory rate Its temperature was 106.8° F The rabbit was killed and its brain carefully removed with sterile precautions A part of the brain was placed in Zenker's acetic fluid, a second portion was cultured in aerobic and anaerobic media, and the remaining bit was ground up in sterile physiological saline solution and the brain suspension was inoculated into the scarified left cornea of a guinea pig This animal developed a typical keratitis and on the fourth day after inoculation showed characteristic signs of encephalitis It was then killed and its brain was placed in Zenker's acetic fluid

Rabbits Numbers 3 and 4 both developed keratitis within twenty-four hours of inoculation and showed definite signs of encephalitis on the seventh day. At that time they were killed and their brains were placed in Zenker's acetic fixing fluid. Histological sections stained with Mallory's eosin-methylene blue stain were made of the fixed tissues. Examination of these sections showed in each instance the severe, infiltrative meningoencephalitis and the acidophilic intranuclear inclusions which are characteristic of herpetic encephalitis in laboratory animals.

Miss C. W. On January 4th, the top of a tense vesicle was removed and was rubbed on the scarified cornea of a rabbit. By January 6th a definite keratitis was present and on the following day the animal was killed and the infected cornea removed. Part of this cornea was placed in Zenker's acetic fluid and the remainder was used to inoculate the scarified cornea of a second rabbit. This animal developed keratitis within two days and showed signs of encephalitis on the eighth day, at which time it was killed and its brain placed in Zenker's acetic fluid. Histological sections stained with Mallory's eosin-methylene blue stain were made of the fixed tissues. The cornea of the first rabbit showed a severe infiltrative keratitis with rare intranuclear inclusion bodies. Evidently the process had progressed beyond the stage most favorable for the finding of inclusion bodies. A severe meningoencephalitis was present in the second rabbit and numerous brain cells showed the acidophilic intranuclear inclusion bodies which are considered to be typical of herpetic infection.

The clinical course of herpetic stomatitis and pharyngitis

In all of our cases the onset was slow and the first symptom observed was either a sore mouth or sore throat. Shortly after the onset, headache and malaise accompanied by sensation of "chills and fever" appeared. Sweating was often marked. It was not possible in any of our cases to determine the relation of the appearance of the herpetic lesions to the onset of symptoms. Nausea and vomiting did not occur. As the disease progressed the symptoms became more intense. In all three individuals generalized aching was present at one time or the other. Two patients had a definite fever when admitted to the Ward. These two also had labial herpes and the lesions were widespread in their mouths. None of our cases developed skin lesions. All had white blood cell counts below 8,000 on entry. The differential formulae were normal. Treatment other than palliative seemed to be of little value as the disease is self limited. None of our patients gave a history of susceptibility to herpes and none have had a recurrence of the infection.

We were able to isolate a virus which produced the typical clinical syndromes of herpetic keratitis and encephalitis in rabbits and guinea

pigs from the vesicles in two of three patients Histological preparations of the corneae and brains from the infected animals showed the acidophilic intranuclear inclusions which are considered to be characteristic of herpetic infections

It is very likely that herpetic affections of the mouth and throat are more common than we suspect It seems curious that a disease apparently so well known and so frequently recognized in the last century should have become rare It is probable that if more attention is paid to the clinical appearance of "sore throats," many of them will be recognized as being herpetic in origin

SUMMARY

Three cases of herpetic pharyngitis and stomatitis have been described and discussed A virus corresponding to the virus of herpes simplex was isolated from the lesions in two of the three cases

BIBLIOGRAPHY

- 1 Gubler, A, Bull Soc méd d hôp de Paris I Serie Tome I, 1858, pp 386-413 Memoire Sur L'Herpes Guttural (Angine Couenneuse Com mune) Et Sur L Opthalmie Due A L Herpes De La Conjonctive
- 2 Trousseau A, Clinique Médicale Tome I, Paris, 1861, pp 431-440 Angines Couenneuses
- 3 Savage, G H, Lancet 1883, 1, 95 Epidemic of Herpetic Fever
- 4 Youmans, J B, South M J 1932, xxv, 228 Herpetic Fever with Stomatitis Report and Discussion of a Case in which the Virus was Isolated

STUDIES UPON THE NASAL SECRETIONS I THE CELLULAR CONTENT OF THE NASAL SECRETIONS IN ACUTE DISEASE OF THE RESPIRATORY TRACT

By PERRIN H LONG, ELEANOR A BLISS AND HARRIET M CARPENTER

(From the Laboratory of the John J Abel Fund for Research on the Common Cold and from the Department of Medicine Johns Hopkins University Baltimore)

(Received for publication July 31 1933)

Although common colds are probably the most frequent ailments from which we suffer, very few studies have been made upon the changes that take place in the nasal mucous membranes during acute upper respiratory infections In 1885 MacKenzie (1) first described the histological appearance of the nasal mucous membranes during acute coryza He found an intense engorgement of the cavernous tissue which was especially well marked over the lower halves of the middle and posterior portions of the inferior turbinates with rupture of the vessels Along the inner walls of the dilated spaces were congregations of lymphoid cells and in some of them collections of fibrinous exudate A moderate cellular infiltration was present below the basement membrane

Forty five years after this report, Hilding (2) published his observations upon the histopathology of the nasal mucous membranes in "colds" He found that "the pathologic process is that of a mucous membrane inflammation showing rather marked tissue changes, including the loss of many of the surface cells and a proliferative reaction in the submucosa The epithelium is regenerated and repaired by the growth and multiplication of the stellate cells normally found deep in the epithelium"

While studying the nasal secretions, Hilding noted that the cellular content changed rapidly Early in colds the secretion was watery and contained few cells Ciliated epithelial cells appeared during the first twenty four hours and became more numerous by the second day The deeper epithelial cells, polyblasts and polymorphonuclear cells appeared in the secretions on the second day and rapidly became numerous Within a few days the polymorphonuclears made up most of the cellular content of the secretions

Hilding did not believe that the cellular content of the secretions or the pathological picture in the nasal mucous membranes was related to any one causal agent in colds, but interpreted the observed changes as a response to any one of or a combination of etiological factors

D and R Thomson (3) have briefly described nasal secretions from cases of acute watery catarrh Their observations are of interest chiefly

assez intense, ainsi que d'un mal de gorge les deux amygdales sont tuméfiées, rouges et ne tardent pas à présenter des surfaces circulaires ou irrégulièrement configurées, semblables à des ulcérations superficielles couvertes d'une exsudation plastique grisâtre ou jaunâtre, souvent cette dernière lésion est unilatérale. En même temps il apparaît sur les lèvres une éruption d'herpès ordinairement groupée en grand partie vers l'une des commissures. Tel est aussi le type consacré par la description classique. Eh bien, dans mon opinion, ces ulcérations tonsillaires doivent être considérées comme de l'herpès labialis sur une surface muqueuse."

Trousseau (2) devoted an entire clinic to the description of herpetic pharyngitis. His description of the individual lesions is classical. "On voit en effet des taches blanches, entourées d'une aréole inflammatoire assez étendue, et dont le volume varie depuis le gros d'un grain de millet jus qu'à celui d'un pois. Ces taches laissent à leur place des ulcérations superficielles dont les bords peuvent néanmoins être saillants en raison du gonflement oedémateux des tissus environnants envahis par l'inflammation."

In 1881 James Wilson in his book on "The Continued Fevers" describes simple continued fever as "a fever not due to any specific cause, usually of short duration, lacking the distinguishing characteristics of other fevers, and rarely fatal in temperate climates. The eruption of herpes upon the lips and nose is so common at the close of simple continued fever that this disease by some persons has been called Herpetic Fever."

Two years later Savage (3) reported an epidemic of herpetic fever which occurred in an English boys' school. The clinical picture of the disease in this epidemic was characterized by a slow onset accompanied by headache, lassitude, chilliness, fever, irritability, malaise and vomiting. After twenty-four hours, patches of herpetic lesions appeared about the lips or lobes of the ears. In two individuals vesicles appeared on the lower extremities. With the appearance of the vesicles the patients complained of sore throats. Frequently herpetic lesions were present in the throat. By the fourth day the temperature was normal and the patients were convalescent. Thirty-nine boys were affected during a two-week period.

In recent years, despite Trousseau's remark that "Au jour d'hui qu'on est suffisamment averti, il est peu de médecins qui n'aient en l'occasion d'en observer des exemples," very little can be found in medical literature regarding the clinical course of localized and generalized herpetic infections of the simplex type. In 1932 Youmans (4) described an individual ill from herpetic fever with stomatitis. He was successful in isolating the virus of herpes simplex from the mouth lesions in this patient, thus for the first time demonstrating the essential etiologic character of the disease.

In this report we wish to discuss three cases of herpetic infections in the mouth, two of which were accompanied by fever. From two of the individuals the virus of herpes simplex was recovered.

CASE HISTORIES

Miss C K J H H 40611, a student, aged 23 entered the Medical Clinic on December 9, 1931, complaining of a "sore throat and swollen tender gums" of three days duration. Her past and family history was unimportant. Three days before coming to the clinic the patient had spent the week end in New York and while riding to Baltimore on the train, she noticed that her throat was sore. Upon the following day she experienced "chills and fever." The soreness in the throat increased and the gums became tender and swollen. Headache appeared and some generalized aching was present. On December 8th the symptoms were more marked and the throat affection was so severe that swallowing became very difficult. She was admitted to the Isolation Ward on the following morning.

Physical examination The patient was undernourished and seemed quite uncomfortable. The temperature was 101.6° F, the pulse 112, and the respirations were 20. The skin was warm and moist, the ears, eyes and nose were normal. The lips were dry and cracked and just to the right of the midline was a small, crusted, superficial ulcerated lesion. The gums were tender, swollen and spongy and there were numerous small superficial lesions capped with white membranes on the tooth gum margins. These bled freely when slightly traumatized. Near the tip of the tongue were several small vesicles as well as upon the hard palate. The tonsils had been cleanly removed, but the soft palate, uvula pillars and posterior pharyngeal wall were intensely inflamed and moderately swollen. The lymphoid follicles of the posterior pharyngeal wall were markedly hypertrophied and there were numerous small superficial vesicular lesions over the posterior pharyngeal wall. The regional lymph nodes were enlarged and slightly tender as were also the axillary glands. The remainder of the physical examination was essentially normal.

Laboratory data Urine normal. Red blood cells 4.4 million. Hemoglobin 90 per cent. White blood cells 4,560. Differential count—Polymorphonuclears 72.5, lymphocytes 12.5, monocytes 15. Wassermann reaction negative. Throat cultures (7), staphylococcus aureus. Throat smears—no Vincent's organisms. Blood culture negative.

Impression It was thought on admission to the Ward that the patient was suffering from an acute septic sore throat and an aphthous stomatitis. The possibility of a Vincent's infection was considered, but at no time were Vincent's organisms demonstrated.

Course The temperature remained elevated on the day after admission and the total white blood cells numbered 6,000. The patient complained of a severe sore throat. The mouth lesions were unchanged. On December 11th the temperature was lower and the white blood cells totaled 5,950. The white membranes capping the vesicles were beginning to slough off, leaving clean superficial ulcers. On December 12th the temperature fell to normal and there was a marked symptomatic improvement. White blood cells 3,750. Treatment of the ulcers by topical application of 2 per cent gentian violet was instituted. During the next few days the patient improved and by December 18th her mouth and throat were free from ulcerated areas. On December 22nd she was discharged well, and as far as we know, she has never had a recurrence of this affection.

Mr M T, J H H 46235, a white, night club saxophone player, aged 25 years, entered the Medical Clinic on November 4th, 1932, complaining of a "sore mouth" of six days duration. His past and family history was unimportant. Five days before coming to the hospital the patient felt "feverish" and thought that his tongue and gums were dry and swollen. A moderately severe headache was present. By evening he developed definite soreness of the throat, tongue and gums. On the following day he felt "grippy" and suffered from generalized aching and malaise. By the next day his throat was so sore and he felt so seedy that he took to his bed. Profuse sweats occurred, his neck became swollen and tender and his headaches more severe. He suffered from insomnia and on the night before entry he was disoriented. All of his symptoms increased in intensity and he came to the hospital.

Physical examination The patient was well developed and well nourished, but apparently was very uncomfortable. The temperature was 100° F, the pulse 96, and the respirations 22. The skin was hot and dry, the ears, eyes and nose were normal. The lips were dry, fissured and scaly. Several small superficial crusted ulcers were present on the outer dry surface of the lips. The inner surface of the lips, the buccal mucous membranes, and the gums were dull red in color. Many deflated vesicles capped with dead white skin were seen on these surfaces. The gums bled when slightly traumatized. The tongue was beefy red and was covered with numerous vesicles. Many similar lesions were seen on the hard palate. The soft palate, uvula, pillars, and posterior pharyngeal wall were moderately inflamed and slightly swollen. On the soft palate, uvula and right posterior pillar there were small vesicles surrounded by a small area of intense hyperemia and capped with a dull chalk-white tense membrane. These collapsed completely when the vesicular fluid was released. The regional and anterior cervical lymph nodes were enlarged and tender. Otherwise the physical examination was normal.

Laboratory data Urine normal. Red blood cells 4.6 million. Hemoglobin 106. White blood cells 6,880. Differential count—polymorphonuclears 62, eosinophiles 1, basophils 1, lymphocytes 34, monocytes 2. Wassermann reaction negative. Throat smears—no Vincent's organisms. Throat culture—staphylococcus aureus.

Impression Because of the presence of typical herpetic lesions upon the lips and the vesicular character of the eruptions in the mouth, it was thought that this patient was suffering from herpetic stomatitis and pharyngitis.

Course On the day following the patient's admission to the hospital, his temperature was normal, but there was no change in the condition of the mouth. The mouth lesions were treated with topical applications of gentian violet. On November 8th the white cells numbered 7,700. The lesions were slowly resolving, but enough discomfort persisted to prevent the taking of solid food. By November 11th the lesions had practically disappeared and the patient was discharged. There has been no recurrence of the herpes up to the present time.

Miss C W, J H H 46136, a student nurse, aged 22, entered the Medical Clinic on January 2nd, 1933, complaining of a "sore throat" of two days duration. With the onset of this affection she suffered from "chills and fever," headache and anorexia. On the following day she felt weak and experienced generalized aching. Profuse sweats occurred during the night. On the day of admission malaise was present and the glands in her neck were enlarged and tender.

Physical examination The patient was well nourished and seemed com-

fortable The temperature, pulse and respirations were normal A faint, generalized erythema was present The ears, eyes and nose were normal, as were also the lips gums, buccal mucous membranes and tongue A discrete vesicular eruption was present over the left anterior pillar and over a right tonsillar tag The vesicles ranged in size from two to four millimeters and were surrounded by small areas of very hyperemic mucous membrane The entire throat was mildly erythematous and the lymphoid follicles on the posterior pharyngeal wall were hypertrophied The regional lymphnodes were moderately enlarged and tender Otherwise the physical examination was normal

Laboratory data Urine normal Red blood cells 4.9 million Hemoglobin 100 White blood cells 7,050 Differential count—polymorphonuclears 65, eosinophiles 1 basophils 1, lymphocytes 29, monocytes 4 Wassermann reaction negative Throat culture—staphylococcus albus and aureus

Impression Herpetic pharyngitis

Course The temperature, pulse and respirations remained normal throughout the patient's stay in the hospital By January 6th the throat had improved and on the following day the patient was discharged from the hospital No local treatment was used in this instance There has not been any recurrence of the affection to date

Experimental studies

Miss C K No studies were made because the nature of the throat affection was not suspected until after the vesicles had disappeared

Mr M T Upon November 4th, the cap of a tense vesicle was removed with biting forceps and this material was rubbed into the scarified left corneae of two rabbits Within twenty four hours a localized injection of the sclera was observed and after a short time one could see minute vesicles along the lines of scarification with the aid of a hand lens By November 7th a full blown, steamy keratitis was manifest in both animals At this point, rabbit Number 1 was killed, the left cornea was removed and then ground in a sterile mortar with a small amount of sterile physiological saline solution The left scarified corneae of rabbits Numbers 3 and 4 were inoculated with the ground material

On the 12th of November rabbit Number 2 was hypersensitive and circled definitely to the left Three days later this animal was extremely hypersensitive, circled to the left, showed marked salivation and retention of urine, ran blindly into objects and had an accelerated respiratory rate Its temperature was 106.8° F The rabbit was killed and its brain carefully removed with sterile precautions A part of the brain was placed in Zenker's acetic fluid, a second portion was cultured in aerobic and anaerobic media, and the remaining bit was ground up in sterile physiological saline solution and the brain suspension was inoculated into the scarified left cornea of a guinea pig This animal developed a typical keratitis and on the fourth day after inoculation showed characteristic signs of encephalitis It was then killed and its brain was placed in Zenker's acetic fluid

Rabbits Numbers 3 and 4 both developed keratitis within twenty-four hours of inoculation and showed definite signs of encephalitis on the seventh day. At that time they were killed and their brains were placed in Zenker's acetic fixing fluid. Histological sections stained with Mallory's eosin-methylene blue stain were made of the fixed tissues. Examination of these sections showed in each instance the severe, infiltrative meningoencephalitis and the acidophilic intranuclear inclusions which are characteristic of herpetic encephalitis in laboratory animals.

Miss C. W. On January 4th, the top of a tense vesicle was removed and was rubbed on the scarified cornea of a rabbit. By January 6th a definite keratitis was present and on the following day the animal was killed and the infected cornea removed. Part of this cornea was placed in Zenker's acetic fluid and the remainder was used to inoculate the scarified cornea of a second rabbit. This animal developed keratitis within two days and showed signs of encephalitis on the eighth day, at which time it was killed and its brain placed in Zenker's acetic fluid. Histological sections stained with Mallory's eosin-methylene blue stain were made of the fixed tissues. The cornea of the first rabbit showed a severe infiltrative keratitis with rare intranuclear inclusion bodies. Evidently the process had progressed beyond the stage most favorable for the finding of inclusion bodies. A severe meningoencephalitis was present in the second rabbit and numerous brain cells showed the acidophilic intranuclear inclusion bodies which are considered to be typical of herpetic infection.

The clinical course of herpetic stomatitis and pharyngitis

In all of our cases the onset was slow and the first symptom observed was either a sore mouth or sore throat. Shortly after the onset, headache and malaise accompanied by sensation of "chills and fever" appeared. Sweating was often marked. It was not possible in any of our cases to determine the relation of the appearance of the herpetic lesions to the onset of symptoms. Nausea and vomiting did not occur. As the disease progressed the symptoms became more intense. In all three individuals generalized aching was present at one time or the other. Two patients had a definite fever when admitted to the Ward. These two also had labial herpes and the lesions were widespread in their mouths. None of our cases developed skin lesions. All had white blood cell counts below 8,000 on entry. The differential formulae were normal. Treatment other than palliative seemed to be of little value as the disease is self limited. None of our patients gave a history of susceptibility to herpes and none have had a recurrence of the infection.

We were able to isolate a virus which produced the typical clinical syndromes of herpetic keratitis and encephalitis in rabbits and guinea

pigs from the vesicles in two of three patients. Histological preparations of the corneae and brains from the infected animals showed the acidophilic intranuclear inclusions which are considered to be characteristic of herpetic infections.

It is very likely that herpetic affections of the mouth and throat are more common than we suspect. It seems curious that a disease apparently so well known and so frequently recognized in the last century should have become rare. It is probable that if more attention is paid to the clinical appearance of "sore throats," many of them will be recognized as being herpetic in origin.

SUMMARY

Three cases of herpetic pharyngitis and stomatitis have been described and discussed. A virus corresponding to the virus of herpes simplex was isolated from the lesions in two of the three cases.

BIBLIOGRAPHY

- 1 Gubler, A, Bull Soc méd d hôp de Paris I Serie, Tome I, 1858, pp 386-413. Memoire Sur L'Herpes Guttural (Angine Couenneuse Commune) Et Sur L'Ophtalmie Due A L'Herpes De La Conjonctive.
- 2 Trousseau A, Clinique Médicale Tome I, Paris, 1861, pp 431-440. Angines Couenneuses.
- 3 Savage, G H, Lancet 1883, i, 95. Epidemic of Herpetic Fever.
- 4 Youmans, J B, South M J 1932, xlv, 228. Herpetic Fever with Stomatitis. Report and Discussion of a Case in which the Virus was Isolated.

STUDIES UPON THE NASAL SECRETIONS I THE CELLULAR CONTENT OF THE NASAL SECRETIONS IN ACUTE DISEASE OF THE RESPIRATORY TRACT

BY PERRIN H LONG, ELEANOR A BLISS AND HARRIET M CARPENTER

(From the Laboratory of the John J Abel Fund for Research on the Common Cold and from the Department of Medicine Johns Hopkins University Baltimore)

(Received for publication July 31, 1933)

Although common colds are probably the most frequent ailments from which we suffer, very few studies have been made upon the changes that take place in the nasal mucous membranes during acute upper respiratory infections. In 1885 MacKenzie (1) first described the histological appearance of the nasal mucous membranes during acute coryza. He found an intense engorgement of the cavernous tissue which was especially well marked over the lower halves of the middle and posterior portions of the inferior turbinates with rupture of the vessels. Along the inner walls of the dilated spaces were congregations of lymphoid cells and in some of them collections of fibrinous exudate. A moderate cellular infiltration was present below the basement membrane.

Forty five years after this report, Hilding (2) published his observations upon the histopathology of the nasal mucous membranes in "colds." He found that "the pathologic process is that of a mucous membrane inflammation showing rather marked tissue changes, including the loss of many of the surface cells and a proliferative reaction in the submucosa. The epithelium is regenerated and repaired by the growth and multiplication of the stellate cells normally found deep in the epithelium."

While studying the nasal secretions, Hilding noted that the cellular content changed rapidly. Early in colds the secretion was watery and contained few cells. Ciliated epithelial cells appeared during the first twenty four hours and became more numerous by the second day. The deeper epithelial cells, polyblasts and polymorphonuclear cells appeared in the secretions on the second day and rapidly became numerous. Within a few days the polymorphonuclears made up most of the cellular content of the secretions.

Hilding did not believe that the cellular content of the secretions or the pathological picture in the nasal mucous membranes was related to any one causal agent in colds, but interpreted the observed changes as a response to any one of or a combination of etiological factors.

D and R Thomson (3) have briefly described nasal secretions from cases of acute watery catarrh. Their observations are of interest chiefly

because of their description of certain round bodies which they saw in the cytoplasm of squamous epithelial cells. Attention is called to the resemblances of these bodies to the guarnieri bodies found within the epithelial cells in vaccinia and variola.

During the past two years we have been studying the cellular content of the nasal secretions in the course of common colds in an attempt to determine the types of cellular response, the nature of the cells, and if possible, the underlying factors which govern the appearance of the various cellular constituents of the nasal secretions in acute upper respiratory tract disease. This communication describes our observations upon the types of cellular response found in the nasal secretions in disease of the upper respiratory tract.

MATERIAL AND METHODS

The subjects utilized in this study were medical students and laboratory workers in the Johns Hopkins University Medical School. A definite attempt was made to obtain the nasal secretions at the beginning and at frequent intervals during the course of the colds. The secretions were gained by having the subject blow his nose upon a clean piece of non-absorbent brown paper and from the nasal discharge preparations were made upon glass slides covered with dried neutral red and Janus green for "supra vital" studies. Permanent preparations were made by evenly smearing the discharge upon clean glass slides and permitting it to dry in the air. The secretions were also observed as to color, consistency and amount.

The "supra vital" preparations were examined immediately in a warm box at 37° C and the percentage of the different types of cells was determined. The air dried smears were subsequently stained with thionin blue and were studied for the presence of bacteria and bacterial phagocytosis. Other air dried slide preparations were stained with either Wright's stain or Mallory's eosin-methylene blue stain and were studied for cell types and for the possible presence of intracellular inclusion bodies. No attempt was made to determine the total number of cells in the specimens of nasal secretions.

We have had the opportunity of examining 302 specimens of nasal secretions in the various stages of 98 colds. The majority of the examinations were made in the first week of the infection. We have been unable to obtain what we considered to be normal nasal secretions for control studies, so we have therefore used the nasal discharge from individuals suffering from acute hay fever to control our observations upon the secretions obtained from persons ill with "colds."

EXPERIMENTAL OBSERVATIONS

In the early stages of colds one commonly finds polymorphonuclear leukocytes, macrophages (monocytes and clasmatocytes), squamous and

columnar epithelial cells, eosinophilic leukocytes, lymphocytes and red blood corpuscles present in the nasal secretions. The cellular response (in the nasal discharge) during the first forty eight hours of a cold may be of three types. In the first type the majority of the cells are macrophages and epithelial cells, in the second type polymorphonuclear leukocytes, and in the third eosinophilic leukocytes. Striking examples of these three types are not commonly encountered as most of the samples of early nasal secretions showed cellular contents which were gradations between the extremes. The three types of response together with the average are shown in Charts 1, 2, 3 and 4.

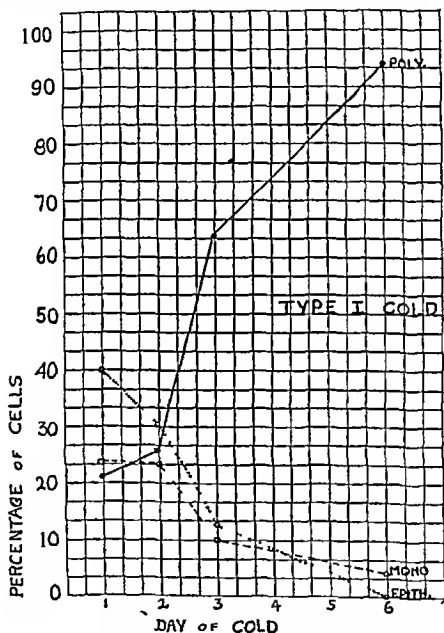


CHART I A COMMON COLD SHOWING A PRIMARY MACROPHAGE EPITHELIAL CELL RESPONSE IN THE NASAL SECRETIONS

When the macrophage epithelial cell type of response is present the secretions contain many macrophages and epithelial cells. The macrophages are easily distinguished in the "supra vital" preparations, but at times the classification of the epithelial cells is difficult because of the bizarre forms which are seen. It is common to find the upper half of the

cells pinched off and then one sees curious globular ciliated cells which resemble bursting grenades. Again one may observe elongated, rounded end, finely granular cells with no visible nuclear structure which may or may not contain vacuoles holding the neutral red and which may or may not have cilia. We think that these cells represent degenerated ciliated columnar epithelial cells. The amount of neutral red taken in by the columnar cells varies from none to 7 or 8 vacuoles filled with this

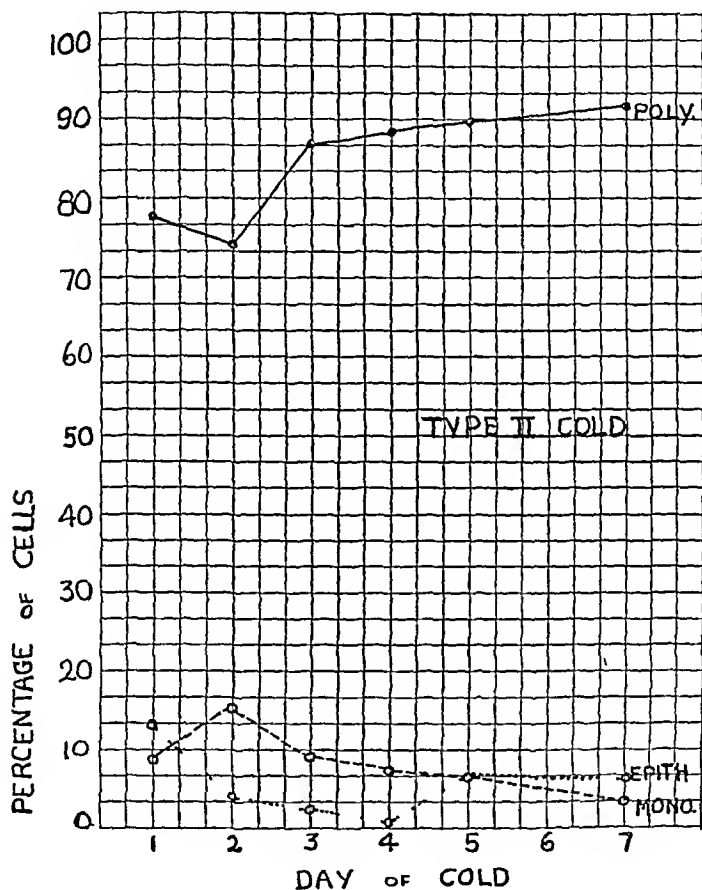


CHART II A COMMON COLD SHOWING A PREDOMINANT POLYMORPHONUCLEAR LEUKOCYTE RESPONSE IN THE NASAL SECRETIONS

dye. The recognition of the squamous epithelial cells from the posterior rhinopharynx and vestibule offers no difficulties.

An evaluation of the viability of the ciliated columnar epithelial cells upon the basis of motility of the cilia and staining of the nucleus has been attempted without much success. In many preparations the cilia were highly motile, in others non-motile. We suspect that in most instances these cells are alive in the secretions, but that due to unavoid-

able crushing during the preparation of the supra vital slide the cilia are so injured that their lack of motility loses its value as a criterion of viability

The macrophages generally appeared to be alive and always showed various amounts of phagocytosed material. We were unable to determine the exact mechanism which regulated the degree of phagocytosis, but we believe that it depended upon the amount of cellular debris present

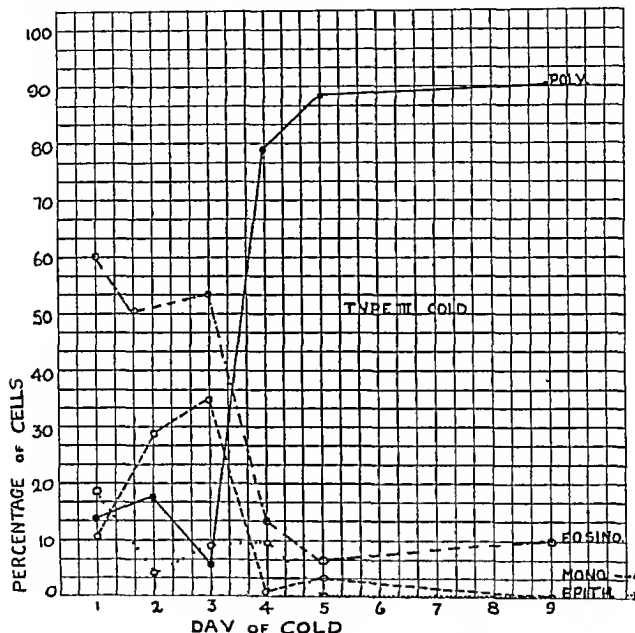


CHART III A COMMON COLD SHOWING A PRIMARY EOSINOPHILE LEUKOCYTE RESPONSE IN THE NASAL SECRETIONS

and not upon the bacterial content of the secretions. Generally, in the macrophage epithelial cell type of response occasional lymphocytes and eosinophiles were present. Red blood cells were encountered in the majority of specimens but a grossly bloody specimen was rarely encountered.

This type of response seldom lasted more than forty eight hours. By the beginning of the third day of the cold the polymorphonuclear leukocytes were abundant and by the fourth day their percentage out-

numbered that of all of the other types of cells. Twenty-three of the ninety-eight specimens of nasal secretions showed this type of response at the first examination.

In the second type of response the percentage of polymorphonuclear leukocytes was always elevated from the first and earliest possible examination. These cells remained predominant throughout the entire course of the cold. Thirty-three of the ninety-eight specimens of nasal

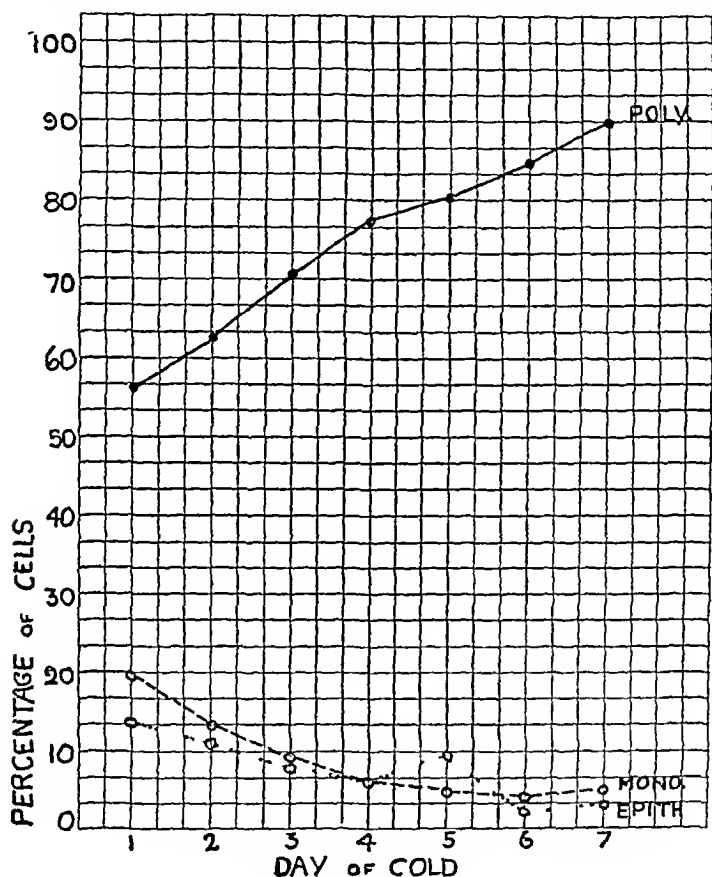


CHART IV THE AVERAGE PREDOMINANT CELLULAR RESPONSE AS DETERMINED IN A STUDY OF THE NASAL SECRETIONS IN 98 COLDS

secretions showed 70 per cent or more polymorphonuclear leukocytes at the first examination. Thirty-nine specimens fell in between these two types of response in that the percentage of polymorphonuclears ranged between 49 and 70 in the initial specimens.

There were three interesting examples of a variation from the general types of cellular responses seen in the nasal secretions in colds. This variation occurred in individuals who gave definite histories of having repeated attacks of pollen hay fever during the past years. In these

individuals there was a marked outpouring of eosinophilic leukocytes into the nasal secretions, which persisted throughout the early stages of the cold. The eosinophiles replaced the polymorphonuclear leukocytes to a large extent without disturbing the macrophage epithelial cell formula. In each instance in which this was encountered the differential white blood cell count was well within normal limits.

It has long been known that in individuals who suffer from hay fever, the nasal submucosal tissues are heavily infiltrated with eosinophiles and that this infiltration persists during the intervals when the acute allergic process has abated. Hence it is not surprising that these individuals should react to an acute infection of the nasal mucous membranes with an eosinophilic response in their nasal secretions.

In our control group of individuals suffering from acute hay fever we invariably found a high percentage of eosinophile leukocytes in the nasal secretions. However, it was interesting to note that macrophages and epithelial cells were present in these secretions in a percentage that is commonly found in colds.

Many of the air dried smears of the nasal secretions were stained with eosin methylene blue and the cells were searched for the presence of intranuclear inclusion bodies. In several instances homogeneous acidophilic bodies were seen in the cytoplasm of columnar epithelial cells in the secretions from early colds. These closely resembled inclusion bodies but due to the lack of control studies upon normal epithelial cells we feel that the assumption that these acidophilic bodies are inclusion bodies would be unwarranted at the present time.

DISCUSSION

It is our opinion that in general our findings confirm the observations of Hilding (2). Undoubtedly the first changes taking place in the nasal mucous membranes during a cold must be an intercellular edema with congestion and an infiltration of macrophages or polymorphonuclear leukocytes. The epithelial cells become swollen and soon detach themselves from the submucosa and are found free in the nasal secretions. The infiltrating macrophages and polymorphonuclear leukocytes either actively wander into the secretion or are passively swept out of the tissue spaces by the edema fluid.

Within forty-eight to seventy-two hours the polymorphonuclear cells become the predominant cell in the nasal secretion and the secretion tends to change from a watery type to a mucopurulent type of discharge, and remains so throughout the remainder of the infection until the stage of crusting is over and the nasal mucous membrane has regenerated. It is well known that a monocytic tissue response is characteristic in certain virus diseases and we feel that the outpouring of monocytes seen in the nasal secretions in early colds may represent a response to the filterable agent of "common colds."

We realize that it is dangerous to argue by analogy, but nevertheless, we would like to call attention to the similarity of the changes taking place in the nasal mucous membranes in colds, to those taking place in the skin during vaccinia. There is first, the stage of engorgement of the nasal mucous membranes which resembles the formation of the vaccine papule. Then comes the tissue edema and destruction of the nasal mucous membrane with an outpouring of fluid containing monocytes which corresponds to the formation of the vesicle filled with fluid in which the mononuclear elements predominate. In the third stage of the cold the secretions have a high polymorphonuclear leukocyte content as does the fluid in the pustule of vaccinia, and finally in both diseases the last stage is characterized by crusting. Here the analogy ends because in colds the mucous membrane is restored while in vaccinia a scar generally results.

CONCLUSIONS

There are two main types of cellular response found in the nasal secretions early in the course of colds. In the first type the macrophages and epithelial cells predominate and in the second the polymorphonuclear leukocytes predominate. Variations between these two types are commonly seen. By the third day of a cold the polymorphonuclear leukocytes become the chief cellular constituent of the nasal secretions. A third type of cellular response in colds in which eosinophiles predominate has been described. It is thought that these observations upon the cellular content of the nasal secretions in colds lend further evidence to the belief that a filterable agent is the cause of acute common colds.

BIBLIOGRAPHY

- 1 MacKenzie, J. N., *New York State J. Med.*, 1885, xlii, 212. A Contribution to the Pathological Histology of Acute and Chronic Coryza.
- 2 Hilding, A., *Arch. Otolaryng.*, 1930, xii, 133. Common Colds.
- 3 Thomson, D., and Thomson, R., *The Common Cold* (Ann. Pickett-Thomson Research Lab., 8). Baillière, London, 1932.

MAGNESIUM METABOLISM IN HYPERPARATHYROIDISM

BY HAROLD A. BULGER AND FLORENCE GAUSMANN

(From the Department of Internal Medicine, Washington University Medical School and Barnes Hospital, St. Louis)

(Received for publication August 7, 1933)

In an earlier, rather extensive, publication in this journal on the "Functional Pathology of Hyperparathyroidism" (1) no mention was made of magnesium metabolism. Data have now been collected permitting a contribution to this phase of the subject. The methods of study were the same as described in the previous paper (1). Magnesium determinations were made by the procedure outlined by Briggs (2).

The pharmacological relationship of calcium and magnesium ions and therefore the necessity for optimum concentrations of both in body fluids suggest a close association of the mechanisms regulating their level in the blood. The fact that magnesium phosphate has solubility properties similar to calcium phosphate and the fairly constant amount of magnesium in bone hint at a specific biological relationship of their compounds.

It would seem that the parathyroid hormone might have at least an indirect influence on magnesium metabolism. The immediate effect of parathyroid hormone on serum magnesium has been studied in dogs by Scholtz (3) and by Greenburg and Mackey (4). Their investigations showed a slight increase in serum magnesium occurring early and before the rise in calcium. By the time calcium had reached maximum values the serum magnesium had fallen towards normal. Our experiments indicated that more prolonged administration of parathormone to dogs resulted in no striking alterations in serum magnesium (Table I). This was also true with rabbits in which parathormone is not very effective in raising the serum calcium.

TABLE I

The effect of continuous parathormone administration on the serum magnesium of dogs and rabbits

	Serum magnesium	
	Before	After
	mgm. per 100 cc.	mgm. per 100 cc.
Dog No. 1 parathormone, 50 to 300 units daily for 4 days	3.3	3.4
Dog No. 2 parathormone, 50 to 300 units daily for 4 days	3.0	5.5
Dog No. 3 parathormone, 50 to 100 units daily for 10 days	2.2	2.9
Dog No. 4 parathormone, 50 to 100 units daily for 10 days	2.1	2.9
Rabbit No. 1 parathormone, 10 units daily for 10 days	2.6	1.9
Rabbit No. 2 parathormone, 10 units daily for 10 days	3.2	2.8
Rabbit No. 3 parathormone, 10 units daily for 10 days	2.8	2.9

In clinical *hypoparathyroidism* serum magnesium tends to be lower than the average for normal individuals. This is not a constant or striking phenomenon. Representative values in tetany following thyroidectomy are presented in Table II. For comparison with the *hypocalcaemia* of parathyroid tetany this table also includes observations on patients with *hypercalcaemia* in generalized neoplastic disease of bone, a condition which at least simulates hyperparathyroidism. These patients also showed low normal figures for serum magnesium.

TABLE II

Serum magnesium, calcium and phosphorus of cases with hypocalcaemia and cases with hypercalcaemia

Cases with hypoparathyroidism following thyroidectomy				Cases with hypercalcaemia associated with generalized neoplastic disease of bone			
	Serum				Serum		
	Mg	Ca	P		Mg	Ca	P
	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc		mgm per 100 cc	mgm per 100 cc	mgm per 100 cc
<i>Case 11317, Tetany</i>	2.1	8.2	4.4	<i>Case 22810, Metastatic Hypernephroma</i>	1.9	14.9	2.5
	2.6	7.9	4.7		1.9	15.1	2.7
<i>Case 22828, Tetany</i>	1.7	7.6	5.5	<i>Case C S, Multiple Myeloma</i>	2.4	13.5	6.8
	1.7	8.8	3.7		1.8	18.1	6.2
<i>Case 23523, Tetany</i>	1.7	7.8	7.7	<i>Case 27621, Multiple Myeloma</i>	2.7	16.5	4.4
	1.8	5.9	5.9		2.0	16.9	4.9
					1.8	16.8	4.0

In *hyperparathyroidism* one finds evidence indicating more clearly that the parathyroid glands have no direct influence on the level of magnesium in the blood. This is illustrated in the studies of a typical case which will later be reported in detail by Olch. The data are recorded in Table III.

Case 26258 This patient, a woman forty years old, had always considered herself healthy. She was raised in the city. Her food habits appeared to have been quite normal. Diphtheria in childhood was the only serious infectious disease she remembered. She was married when seventeen years old. Her only pregnancy was at eighteen years and was apparently quite normal and was followed by an uneventful puerperium. About this time dental caries began to develop and careful cooperation with her dentist failed to save her teeth. Fillings continued to fall out and caries advanced until finally all her teeth were removed. Her weight was continuously around 125 pounds until her hospitalization.

Six years before admission the patient fell on the floor from the level of a bed and broke a clavicle. Three years later she fell on the ice and broke her left humerus. Two years before admission she developed dull pain above her

TABLE III

Indicating the influence of variations in parathyroid activity on serum magnesium

Case 26253 Hyperparathyroidism A parathyroid adenoma removed January 8 1931	Serum			Date
	Mg	Ca	P	
	mgm per 100 cc.	mgm per 100 cc.	mgm per 100 cc.	
Before operation	1.6	16.2	1.9	Nov 4, 1930
Before operation	1.7	14.0	2.3	Jan 8 1931
One day after removing parathyroid tumor	1.7	9.8	1.7	Jan 9 1931
Four days after removing parathyroid tumor	1.7	8.3	1.7	Jan 12, 1931
Seven days after removing parathyroid tumor	1.8	8.9	2.4	Jan 15 1931
Eleven days after removing parathyroid tumor	1.9	9.6	2.1	Jan 19 1931
Eighteen days after removing parathyroid tumor	2.5	9.5	2.6	Jan 26 1931
Three months after removing parathyroid tumor	2.3	10.7	2.4	Apr 2 1931
One year after removing parathyroid tumor	2.5	10.2	3.6	Dec 18, 1931
Case 35890 Parathormone administration to a young woman normal except for otosclerosis				
Before parathormone	2.2	10.1	3.7	Sept 20, 1932
After 100 units parathormone in 24 hours	2.6	12.5	2.9	Sept 22 1932
After 160 units parathormone in 48 hours	2.7	12.5	2.9	Sept 23, 1932
After 400 units parathormone in 5 days	2.7	13.4	1.5	Sept 26, 1932
After 580 units parathormone in 7 days	2.6	12.8	2.3	Sept 28 1932
36 hours after stopping parathormone	2.5	10.9	2.5	Sept 30 1932
Four days after stopping parathormone	2.5	10.3	3.8	Oct 3 1932
Seven days after stopping parathormone	2.6	10.5	3.3	Oct 6 1932

left knee This was thought to be rheumatism but, when it did not improve after months an x ray was taken Her physician told her there was a lack of calcium in this area and that if she ever broke it healing might not take place The pain gradually improved

The patient then felt perfectly well until June 25 1930, when she stumbled over a hedge and fell on a sidewalk and broke her left femur and right humerus X ray examinations showed these to be pathological fractures through multilocular cystic areas in the bone Further x ray studies showed similar areas in other bones especially in the pelvis and upper dorsal spine X ray also showed shadows in both kidney regions typical of bilateral nephrolithiasis

Physical examination disclosed little for comment except moderate emaciation an upper dorsal kyphosis, hyperactive deep reflexes and a systolic blood pressure of 180 and a diastolic of 100 No tumor in the thyroid region was evident Blood examinations showed high serum calcium, low serum phosphate, a slight anemia normal nonprotein nitrogen and negative Wassermann and Kahn reactions The basal metabolic rate was - 4 per cent Phenolsulphonphthalein excretion was 70 per cent

On October 30 1930 surgical exploration of the thyroid region by Dr I. Y. Olch disclosed a small tumor, about 1.5 by 2 cm. in size in the left lobe After this was removed the serum calcium did not fall and microscopic study showed it to be a foetal adenoma of the thyroid On January 8, 1931, the neck was explored again and a tumor 1.5 by 4 cm. in size was found lying in a

crevice between the oesophagus and spine and attached by a pedicle to the upper pole of the left lobe of the thyroid. After removing this tumor the serum calcium fell to below normal and the patient experienced moderately distressing symptoms of tetany for about ten days.

Striking improvement in this patient's skeletal abnormalities, as evidenced by x-ray examinations, has followed operation. The improvement in her general condition has been less marked. The bilateral nephrolithiasis opposes a satisfactory course. Although there are no local symptoms, hypertension increases steadily and eventual kidney insufficiency is feared. Her general condition will not permit removal of the stones.

While in the hyperparathyroid state the serum magnesium varied from 1.6 to 2.0 mgm per 100 cc. These figures are low but within the range of normal variations. Following the removal of the parathyroid adenoma the serum calcium fell typically to values below normal, but this change in state was not attended by any alteration in the level of serum magnesium. During the three weeks following operation magnesium varied from 1.8 to 2.0. Three months later when the calcium was 10.7 mgm and phosphorus 2.4 mgm the serum magnesium was 2.3 mgm per 100 cc. A year after operation the figures were about the same except that the serum phosphorus had risen to normal.

Studies of magnesium, calcium and phosphorus metabolism of this patient, with clinical hyperparathyroidism, are recorded in Table IV. Period 9, the first included in this table, was preceded by eight similar periods in which only the calcium and phosphorus were determined. As in period 9, they showed the typical negative balance of these elements. A definite tendency to lose magnesium in hyperparathyroidism is indicated by the data in this table. A significant relationship to the hyperparathyroid state is emphasized by the change in the magnesium balance which followed the extirpation of a parathyroid adenoma. As the patient was transformed to relative hypoparathyroidism and the serum calcium fell to below normal, the loss of magnesium shifted to a distinctly positive balance. Magnesium was retained as large amounts of calcium and phosphorus were stored. One year after operation the patient was studied for one period on the same diet she was following at home. This contained large amounts of magnesium, calcium and phosphorus, chiefly from milk. The retention of the three elements was still quite marked.

These modifications of magnesium excretion may be secondary to an effect of the parathyroid hormone on calcium or phosphorus of bone. It is interesting to compare the ratios of the balances of magnesium, calcium and phosphorus with the proportions of these substances in bone. Considering calcium as 100, one may calculate the proportions shown in Table V.

It is apparent that a loss of calcium is accompanied by a loss of magnesium and phosphate and that a retention of calcium is accompanied by a retention of the other two elements as might be expected from the

TABLE IV

Magnesium calcium and phosphorus metabolism of patient 26253 with hyperparathyroidism and the influence of removing the parathyroid adenoma
Also metabolism studies of patient with generalised neoplastic disease of bone and hypercalcaemia. Corresponding serum magnesium calcium and phosphorus figures are recorded in Tables II and III

4 day periods (1930-31)	Period	Magnesium				Calcium				Phosphorus			
		Intake	Output	Urine	Stool	Intake	Output	Urine	Stool	Intake	Output	Urine	Stool
		grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
Case 26253													
November 4 to 7 November 28 to December 1 December 2 to 5 December 6 to 9 December 10 to 13 January 13 to 16 January 17 to 20 January 25 to 28 January 29 to February 1 December 17 to 20	9	1 128	1 444	0 539	0 905	14 41	22 51	2 24	20 27	7 00	8 01	5 02	2 99
	12*	1 065	1 038	0 634	0 404	11 76	7 04	1 79	5 25	7 02	7 86	5 65	2 21
	13*	0 993	1 220	0 106	1 115	9 83	13 21	0 73	12 48	7 20	7 76	4 45	3 31
	14*	1 044	1 136	0 365	0 771	10 29	10 17	0 86	9 31	7 38	6 95	4 88	2 07
	15*	0 986	1 009	0 157	0 852	11 42	13 36	0 79	12 58	6 39	5 92	3 66	2 26
	20	0 808	0 679	0 137	0 542	14 00	8 95	0 09	8 86	3 24	1 21	0 20	1 01
	21	0 883	0 912	0 196	0 716	12 11	10 47	0 04	10 43	3 40	2 02	0 76	1 26
	23	0 846	0 632	0 076	0 556	12 58	7 25	0 08	7 17	3 20	2 28	1 21	1 07
	24	0 827	0 671	0 066	0 605	11 88	3 88	0 09	3 79	3 69	1 74	1 09	0 65
	25	2 414	1 854	0 554	1 300	15 82	7 51	0 07	7 44	11 25	6 87	4 86	2 01
	Case 27621												
January 28 to February 1 February 6 to 9	1	0 468	0 456	0 175	0 281	2 30	3 06	1 12	1 94	2 64	2 61	1 62	0 99
	2	0 400	0 391	0 156	0 235	2 11	4 02	1 50	2 52	2 49	2 88	1 99	0 84

* In periods numbers 12, 13 and 14 irradiated ergosterol (Acterol 20 drops t.i.d.) was administered in period number 15 cod liver oil. Medication may have caused some tendency to retention of calcium and phosphorus

TABLE V
Ratios of magnesium and phosphorus to calcium

	Calcium	Magnesium	Phosphorus
	<i>grams</i>	<i>grams</i>	<i>grams</i>
Approximate proportions in bone*	100	2	50
Loss the month before operation	100	7.4	17.6
Retention the month after operation	100	2.3	31.4
Retention one year after operation	100	6.7	53.8

* Analysis of bone from another case of hyperparathyroidism gave the following figures: Calcium 15.39 per cent, magnesium 0.23 per cent, and phosphorus 6.89 per cent. These would give ratios of 100 : 1.5 : 45.

composition of bone. Significant variations from the ratios in bone do occur but further discussion of the data would not be profitable.

A study of a patient with generalized neoplastic disease of bone and hypercalcaemia is included in Table IV (*Case 27621*). This patient showed a markedly negative calcium balance and a slight loss of phosphorus. Nevertheless, she appeared to be in magnesium equilibrium.

Magnesium metabolism was also studied in experimental hyperparathyroidism. A young woman 27 years old, who appeared normal except for moderate undernutrition and otosclerosis, was given 70 to 100 units of parathormone (Lilly) daily for eight days. Magnesium, calcium, phosphorus, nitrogen and sulphur balances were investigated, before, during, and subsequent to the hyperparathyroid state. The results of four day periods of study are summarized in Table V. During the preliminary observations she demonstrated an ability to retain all elements, probably because of an improved dietary regimen. During the eight days of parathormone administration there was a distinct increase in magnesium output, resulting in an evident negative balance. This was due entirely to a rise in urinary magnesium excretion. The serum magnesium rose slightly. When parathormone was discontinued there was a prompt storing of magnesium but no change in the level in the serum. In these observations the mobilization of calcium and phosphorus was quite typical. The excessive phosphorus excretion was greater than could be accounted for by calcium phosphate liberated and the magnitude of the increased protein metabolism. This surplus phosphorus was more than enough to explain the magnesium loss as magnesium phosphate released from the body. In the recovery period the retention of phosphorus could reasonably be accounted for by magnesium phosphate, calcium phosphates, and protein restored.

The negative nitrogen and sulphur balance caused by parathormone may be related to the local inflammatory and slight general febrile reactions caused by the material injected rather than any specific effect of the hormone itself.

TABLE VI

Magnesium, calcium phosphorus nitrogen and sulphur metabolism in four day periods of patient 35690, showing the effect of administering parathormone for eight days. Parathormone was given through periods number 2 and 3. The amounts of parathormone and corresponding serum magnesium, calcium and phosphorus figures are recorded in Table III.

4 day periods (1932)	Pe riod	Magnesium						Calcium						Phosphorus						Nitrogen						Sulphur																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
		Intake			Output			Intake			Output			Intake			Output			Intake			Output			Intake			Output			Intake			Output																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
		grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams

* By accident the stools of periods numbers 2 and 3 were mixed. For convenience of presentation one half of the total was assumed for each period.

CONCLUSIONS

In the hyperparathyroid state there is a negative magnesium balance. Individuals reverting from hyperparathyroidism to normal or the hypoparathyroid state store magnesium. Little evidence was obtained indicating the parathyroid glands have any direct effect on the level of magnesium in the blood. The influence of the parathyroid hormone on magnesium metabolism may be secondary to its action on calcium or phosphorus.

BIBLIOGRAPHY

- 1 Bulger, H. A., Dixon, H. H., Barr, D. P., and Schregardus, Olive J., *J. Clin. Invest.*, 1930, **1**, 143. The Functional Pathology of Hyperparathyroidism.
- 2 Briggs, A. P., *J. Biol. Chem.*, 1922, **11**, 349. A Colorimetric Method for the Determination of Small Amounts of Magnesium.
- 3 Scholtz, H. G., *Arch. f. exp. Path. u. Pharmakol.*, 1931, **clix**, 233. Notiz über die Wirkung des Parathyreoidea-Hormons auf den Magnesiumgehalt des Blutes.
- 4 Greenburg, David M., and Mackey, Myrtle A., *J. Biol. Chem.*, 1932, **xcviii**, 765. The Effect of Parathyroid Extract on Blood Magnesium.

IMMUNITY IN DIABETES INFLUENCE OF DIABETES ON THE DEVELOPMENT OF ANTIBACTERIAL PROPERTIES IN THE BLOOD

BY RUSSELL RICHARDSON

(From the George S. Cox Research Institute University of Pennsylvania Philadelphia)

(Received for publication August 8 1933)

Notwithstanding the well known association of diabetes and infection we have little definite knowledge of the mechanism by which these two conditions affect each other. Since the days of Claude Bernard, clinicians have viewed with alarm either the appearance of infection during the course of diabetes or, perhaps with less apprehension, the occurrence of diabetes during or following an infection. In considering the coincidence of these disorders one might distinguish between the effect of each upon the other as different though essentially interdependent problems. Various degrees of importance have been accorded to infection in the etiology of diabetes but there is general agreement regarding the harmful effect of infection when occurring during the course of diabetes.

The resistance of the body to infection is attributed in part to the antigen amboceptor complement reactions of the blood. We have no knowledge regarding the influence of diabetes on these reactions nor on the formation of amboceptor or complement. The former may be present in the blood, as in natural immunity, or it may be formed following the inoculation of bacteria into the body, as in acquired immunity. Complement appears to be always present and to react with either type of amboceptor. In this study, complement, natural amboceptor and acquired amboceptor, as factors in the antibacterial power of the blood, have been investigated in diabetic and non diabetic persons to determine whether this part of the protective mechanism of the blood is affected by diabetes.

The complement of the serum was measured in hemolytic and bacteriolytic systems. The bactericidal power of the whole blood was chosen as the best method of determining the native amboceptor (1, 2). The formation of agglutinins following inoculation with *B. typhosus* vaccine was taken as the index of formation of acquired amboceptor.

COMPLEMENT

Method The hemolytic complement was measured in the sheep-rabbit hemolytic system. One half cc amounts of a 2 per cent suspension of washed sheep cells with an excess of amboceptor were taken with

increasing amounts of patients' serum and the total contents of each tube brought up to 3 cc with normal saline. The amount of serum necessary to provide an excess of amboceptor had been determined by preliminary titration. The tubes were then incubated at 37.5° C for 1 hour and the titre read as the smallest amount of serum that caused complete hemolysis. The sera were all fresh, the tests being done about 5 hours after the blood had been collected. On account of the variation in sheep cells, both diabetic and normal sera were set up in every series. Whether the patients were taking insulin was noted.

The patients were selected to represent wide variations of diabetes as regards severity, age, use of insulin and state of nutrition. A blood sugar was done on each blood taken.

The bacteriolytic complement was measured by means of the bacteriolytic action of diabetic and non-diabetic sera on *B. typhosus*. An excess of amboceptor in the form of human serum heated to 56° C for 30 minutes was added to increasing amounts of patients' sera and 0.1 cc of 24 hour broth culture of the organism. After 24 hours the tubes were cultured on blood agar plates and the titre taken as the lowest amount of serum which completely killed the *B. typhosus*.

Results In all, 208 diabetic and 102 non-diabetic sera of patients without infection were examined. Of these, 191 diabetic and 97 non-diabetic were measured in the hemolytic system, while 17 diabetic and 5 non-diabetic individuals were used to determine the bacteriolytic complement. The complement titre of the serum in the non-diabetics varied from 0.020 cc to 0.140 cc with an average of 0.056 cc, while in the diabetics it varied from 0.030 cc to 0.120 cc with an average of 0.052 cc.

In the measurement of bacteriolytic complement similar agreement was found. The titre of both diabetic and non-diabetic sera lay within the same range, namely, between 0.010 cc and 0.075 cc.

Furthermore, diabetic patients with infection showed no change in the amount of their complement titre as compared with non-diabetics with infection. Nineteen of the former and 12 non-diabetics were set up. In each group the titre varied between 0.020 cc and 0.080 cc. The averages for these groups were 0.040 cc and 0.052 cc respectively.

It is evident from these studies that there is no significant variation from normal in the amount of complement present in the blood of patients with diabetes regardless of whether the blood sugar was high or low and whether or not they were taking insulin. The presence of infection did not appear to alter the complement content of the blood.

BACTERICIDAL EXAMINATIONS

Since Nuttall in 1888 demonstrated the bactericidal action of whole blood, two methods of measuring the antibacterial titre have been

commonly selected Todd (1) and others following Wright used defibrinated blood, while Heist (2) used fresh coagulable blood. Both of these methods were employed in the present study with each patient and control. Five organisms were used throughout the entire series of determinations, namely, *B. coli*, *Pneumococcus*, *Staphylococcus aureus*, *Streptococcus hemolyticus* and *Streptococcus viridans*. *B. typhosus* and *B. pyocyaneus* were also included in some experiments. Because of the length of time necessary to complete the work, cultures which had been growing as laboratory strains for some time were taken so that there would be little variation in their behavior. All of the organisms had been in artificial cultivation for at least a year. Transplants were made at frequent intervals on human blood agar and from these, broth cultures were made 18 hours before the test and incubated at 37.5° C. Blood sugar determinations were done on all blood specimens.

The defibrinated blood was used according to the method of Todd as given by Ward (3). Defibrination was done in 150 cc. flasks containing glass beads and leukocyte counts were made in a number of cases before and after shaking in order to determine the loss in leukocytes. Dilutions of 1/10, 1/100, 1/1000, 1/10,000, 1/100,000 and 1/1,000,000 of the 18 hour broth cultures were made, and 0.1 cc. of each dilution was placed in one of a series of small glass tubes. There was then added to each tube 0.5 cc. of defibrinated blood. The tubes were sealed by heat, and placed in a rack attached to an electric motor so as to revolve at the rate of about 6 revolutions per minute. The entire apparatus was placed rotating in the incubator at 37.5° C. At the end of 24 hours the tubes were opened and cultures were made on human blood agar plates. These were incubated for 48 hours and examined for growth. In each test also, human blood agar plates were seeded with 0.1 cc. of the 1/100,000 dilution of each organism and the colonies counted after incubation for 24 hours.

In order to minimize the effect of variations in the number of bacteria present in the 18 hour broth cultures, each determination consisted of one normal and two diabetic persons, except in four instances when only one diabetic was used. As far as possible attempt was made to get a severe and a mild diabetic to compare with each non-diabetic control. Controls were members of the Institute Staff and medical students.

The fresh coagulable blood was used according to the method of Heist (2). Into sterile, cotton stoppered test tubes, 85 X 15 mm., were placed 0.1 cc. of the same dilutions of the five organisms as already described, and these overlaid with 0.5 cc. of freshly drawn blood. The tubes were gently shaken and allowed to stand until coagulated, when they were placed in the incubator at 37.5° C. After 24 hours, cultures were made and examined as in the method with defibrinated blood. Sugar determinations were done on all specimens of blood.

Results The results of the bactericidal tests by the method using coagulable blood are given in Table I. In these are shown the number of diabetic patients whose blood killed the same number of organisms

TABLE I

Comparison of number of organisms killed by diabetic blood with number killed by non-diabetic blood (Coagulable blood method)

Bacteria killed by diabetic blood	Diabetics whose blood killed at least 100 fold less bacteria than non-diabetic control			Diabetics whose blood killed approximately the same number of bacteria as non-diabetic control			Diabetics whose blood killed at least 100 fold more bacteria than non-diabetic control			Total patients
	1 10 000	1 1 000	1 100	1 10	1	10	100	1 000	10 000	
Bacteria killed by non-diabetic blood										
<i>B. coli</i>			2	17	19	8				46
<i>Pneumococcus</i>	2	3	8	12	14	4	1	1	1	46
<i>Staphylococcus aureus</i>		7	5	14	6	7	3	2		44
<i>Streptococcus hemolyticus</i>	3	4	6	10	14	6	1			44
<i>Streptococcus viridans</i>	3	6	3	16	14	2	1	1		46

as the normal control and the number whose blood killed less than or more than the control. This method of reporting the results of the tests was adopted because it seemed the simplest way in which the influence of variation in number of bacteria in the broth cultures could be eliminated. The diabetic bloods were compared only with the normal bloods which had been set up at the same time, with the same broth cultures. The difference between the largest and smallest numbers of bacteria in the series of cultures of any one organism was less than the 10-fold dilution which was used between successive tubes of the determinations, so that even if this variation were not considered, the error would be less than one dilution in the series of 6 tubes.

It will be noted that the distribution of cases shows that diabetic patients in general are less able to kill the test bacteria than the non-diabetic controls. This seems to be especially true with the gram positive cocci against which phagocytosis has been shown to be especially effective. It appears that the diabetic tends to be deficient in whatever antibacterial bodies are naturally present in the blood. From Table II it is evident that there is no significant correlation of distribution of the antibacterial power of the blood with variations in the blood sugar. There seems to be some factor in the diabetic, other than the blood sugar at the time of the determination, which influences the bactericidal effect.

A comparison of results of the methods using defibrinated and coagulable blood gave essentially similar results. The same difference between diabetic and non-diabetic bloods could be recognized in both. The method with coagulable blood generally showed a slightly higher bactericidal titre than did the method with defibrinated blood.

TABLE 11

Comparison of organisms killed by diabetic blood containing over 200 mgm and under 200 mgm of sugar to those killed by non diabetic blood

Bacteria killed by diabetic blood	$\frac{1}{10\ 000}$	$\frac{1}{1\ 000}$	$\frac{1}{100}$	$\frac{1}{10}$	1	10	100	1 000	10 000
Bacteria killed by non diabetic blood									
<i>B. coli</i>									
Under 200 mgm			1	8	12	6			
Over 200 mgm			1	9	7	2			
<i>Pneumococcus</i>									
Under 200 mgm	2	1	5	8	8	1	1	1	
Over 200 mgm		2	3	4	6	3			1
<i>Staphylococcus aureus</i>									
Under 200 mgm		5	3	11	5	4	1	2	
Over 200 mgm		2	2	3	1	3	2		
<i>Streptococcus hemolyticus</i>									
Under 200 mgm	2	3		8	9	4			
Over 200 mgm	1	1	6	2	5	2		1	
<i>Streptococcus viridans</i>									
Under 200 mgm	2	4	2	10	8		1		
Over 200 mgm	1	2	1	6	6	2		1	

Conclusions Diabetic whole blood, regardless of the level of its blood sugar, has in general a weaker bactericidal property than has non-diabetic whole blood as tested by standard methods

ANTIBODY FORMATION

In addition to the presence of complement and natural amboceptor, immunity against bacterial infection is influenced by the ability of the body to form antibodies for specific infections. In order to compare the formation of an acquired antibody in diabetic and non-diabetic persons, the response to typhoid vaccine was investigated.

Methods Three doses, 500 million, 1000 million and 1000 million respectively of *B. typhosus* vaccine¹ were given subcutaneously at intervals of seven days to 41 diabetic and 39 non diabetic persons. Record was kept of the occurrence of local or general reactions in both series. Reactions, if present, being noted as slight, moderate or severe.

The vaccine used was freshly made in order to get the highest possible agglutinative titre in the blood. It was given first to the diabetic patients and later to the non-diabetic controls. There was probably very little difference in the potency of the vaccine in the two series as the last inoculation was given within 8 weeks of the time of making the vaccine.

Blood for determination of agglutinative titre was taken 2 weeks and 10 weeks after the last dose. The titre was determined by Dreyer's (4) macroscopic method, using typhoid antigen killed and preserved by

¹ Furnished through the courtesy of Lederle Laboratories Inc for this work

formalin The same strain of *B typhosus* from which the vaccine was made was procured from the makers for the purpose of making the antigen The same antigen was used throughout the 8 weeks Known positive and negative sera were run with each determination, the positive serum regularly clumping the antigen at a dilution of 1/20,480

The non-diabetic controls consisted of the entering class of nurses at the Methodist Episcopal Hospital in Philadelphia The diabetic patients were ambulatory cases from the clinic, and varied in severity, though none were in such condition that giving the vaccine would be a dangerous procedure It was impractical to get the patients of the same young age group as the controls though most of the former were under 40 years Frequent blood and urine examinations were made, and on the basis of these, as well as of the clinical condition, the patients were divided into three groups, namely, those in good, fair and poor clinical condition The first were those who had always a normal blood sugar with no sugar in the urine and who felt generally well The second group comprised those who were in fair condition and who more or less successfully maintained their diets, their blood sugar levels lay between 140 and 200 mgm per 100 cc, and glycosuria was sometimes present The third were those who always had a blood sugar over 200 mgm and sugar in most, if not all, of the urine specimens

TABLE III

Agglutinative titre of diabetic patients and non-diabetic controls

	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	1/20480
2 weeks after vaccine											
Non-diabetics				1			1	4	6	7	20
Diabetics			1	2	2	6	7	5	10	6	2
Good clinical condition							3	1	1	2	1
Fair clinical condition					1	2	2	3	6	4	
Poor clinical condition			1	2	1	4	2	1	3		1
10 weeks after vaccine											
Non-diabetics					1	2		4	11	19	1
Diabetics	1	4	2	6	6	15	4	4	3		
Good clinical condition			1	1	1	4	1				
Fair clinical condition		1		2	2	9	2	2			
Poor clinical condition	1	3	1	3	3	2	1	1			

Results In Table III are shown the results of the agglutination tests It is quite evident that there is a marked difference in the titre of the diabetic and non-diabetic series at the end of both 2 weeks and 10 weeks Fifty per cent of the non-diabetics agglutinated at a dilution

of 1/20,480 compared with 5 per cent of the diabetics, and 84 per cent of the non diabetics agglutinated at 1/5120 or above, compared with 44 per cent of the diabetics. The persistence of titre of the serum for 8 weeks is much less in the diabetics than in the non diabetics. At the end of this time 81 per cent of the non diabetics were found to agglutinate at a dilution of 1/5120 or more compared with 7 per cent of the diabetics. The effect of the high blood sugar on the formation of antibodies does not appear to be definite, though there is perhaps a slightly less effective formation in the patients whose diabetic condition is described as poor.

The author wishes to acknowledge with thanks many helpful criticisms and suggestions by Dr Herbert Fox and Dr J Harold Austin. Thanks are due also to Miss Elizabeth F Barth, Miss Peggy C Kostal and Miss Jessie Paul for technical assistance.

SUMMARY

Complement in the blood of diabetic patients does not differ in amount from that in the blood of non-diabetics. This is true whether or not infection is present.

The antibacterial power of the blood of diabetic patients as measured by standard methods tends to be less than that of non diabetics.

Diabetic patients are less able than non diabetic controls to form agglutinins following their inoculation with typhoid vaccine.

From these studies it appears that any deficiency in the antibacterial reactions of the blood of the diabetic comes rather from impairment of the amboceptor than from any lack of amount or activity of the complement. This is true of both the natural amboceptor as shown by the bactericidal test, and of the acquired amboceptor, as shown by formation of agglutinins.

BIBLIOGRAPHY

- 1 Todd E W Brit J Exper Path, 1927 viii, 1 A Method for Measuring the Increase or Decrease of the Population of Hemolytic Streptococci in the Blood
- 2 Heist, G D Solis Cohen, S, and Solis Cohen, M, J Immunol, 1918, iii, 261 The Bactericidal Action of Whole Blood with a New Technic for its Determination
- 3 Ward H K J Exper Med, 1930, li, 675 and 685 Observations on the Phagocytosis of the Pneumococcus by Human Whole Blood I and II
- 4 Dreyer G, Gibson A G and Walker, E W A Lancet, 1916, i, 766 Further Remarks on Agglutination Tests in Inoculated Persons and the Influence of Febrile Conditions on Inoculation Agglutinins

A NOTE ON THE CALCULATION OF WATER EXCHANGE

By L H NEWBURGH F H WILEY AND M W JOHNSTON

(From the Department of Internal Medicine Medical School, University of Michigan)

(Received for publication July 13, 1933)

In a recent publication (1), Peters, Kydd, and Laviates have "simplified" the method proposed by Newburgh and Johnston (2) for determining water exchange. Later publications from this laboratory (3), in which it was shown that the calculation of heat production directly from the insensible loss of weight is not accurate, have an important bearing on the suggestion of Peters, Kydd and Laviates who propose to calculate heat production directly from the insensible loss of weight by means of their Equation 2

$$1\ 301\ (I\ L) + 19\ 27 = \text{Cal per hour},$$

where I L expresses insensible loss of weight per hour. This equation is derived from the data of Benedict and Root (4), who demonstrated the fact that the insensible loss of weight is roughly proportional to the heat production in the basal state.

We have found that this relationship is not quantitative for long periods of varying activity during which food is ingested, because the R Q does not remain uniform, as it tends to in the basal state.

The nature of the insensible loss of weight may be expressed algebraically as follows

$$I\ L = H\ O + C\ O_2 - O$$

It is obvious from the above equation that the only factor concerned in the dissipation of heat is the water that leaves the organism as vapor and carries with it 58 cal per gram. We have demonstrated that the heat lost in this way is 24 per cent of the total heat when certain precautions are in effect. Hence, the relationship is one between the vaporization of water and heat rather than between the insensible loss of weight and heat. While the water vapor is a component of the insensible loss of weight, there is no strictly quantitative relationship between the two, as may be seen from the following table

R.Q	Water Vapor grams/4 hrs	I L. grams/24 hrs
1.00	978	1193
0.82	978	1054
0.707	978	940

When the heat production calculated by the method proposed by us is compared with the "simplified" method advocated by Peters and his associates, the following values are obtained

R Q	According to Newburgh et al Cal/24 hours	According to Peters et al Cal/24 hours
1 00	2367	2116
0 82	2367	1833
0 707	2367	1403

Therefore the Equation 2 of Peters, Kydd and Laviertes entails a serious error which limits the value of the subsequent equations that depend upon the validity of this one

We agree with Peters, Kydd and Laviertes that preformed water should not be included in the water balance if one follows the standard practice in regard to balances. However, consideration of preformed water, as employed by Newburgh and Johnston, gives information that could not otherwise be obtained. They desired to secure a precise statement of the expected change in body weight in response to any given diet. It seemed proper to assume that, in the adult subject, there is an optimum value for the total volume of the circulating fluids. The water of the body is either part of this circulating fluid or of the protoplasm. It is generally recognized that, in the absence of disease, the per cent of water in the protoplasm is constant. Hence, any change in body weight that was not explained by change in the mass of protoplasm (i.e. solids plus their associated water), would be attributable to change in the volume of the circulating fluids. This latter phenomenon would then be a departure from the theoretical optimum. To evaluate this response, it was necessary to know how much water was to be apportioned to the gain or loss of protoplasmic solids. This increment of water was accordingly added algebraically to the sources of water, and was called "Preformed Water". Newburgh and Johnston were not concerned with the question whether such water is "free" or "bound."

REFERENCES

- 1 Peters, J. P., Kydd, D. M., and Laviertes, P. H., *J. Clin. Invest.*, 1933, **xii**, 689. A Note on the Calculation of Water Exchange.
Peters, J. P. and Laviertes, P. H., *J. Clin. Invest.*, 1933, **xii**, 695. The Nature of Preformed Water.
- 2 Newburgh, L. H., Johnston, M. W. and Falcon-Lesses, M., *J. Clin. Invest.*, 1929-30, **viii**, 161. Measurement of Total Water Exchange.
- 3 Newburgh, L. H., Wiley, F. H. and Lashmet, F. H., *J. Clin. Invest.*, 1931, **x**, 703. A Method for Determining Heat Production over Long Periods of Time.
- 4 Benedict, F. H. and Root, H. F., *Arch. Int. Med.*, 1926, **lxxxviii**, 1. Insensible Perspiration, Its Relation to Human Physiology and Pathology.

THE RATE OF EXCRETION OF URINE IN SUBJECTS WITH DIFFERENT AMOUNTS OF RENAL TISSUE¹

By DAVID A. RYTAND M D

(From the Department of Medicine, Stanford University School of Medicine San Francisco)

(Received for publication August 9 1933)

In the vast literature on renal function, there is to be found only one method proposed as an indirect means of measuring the mass of functioning kidney tissue, namely, the Addis Ratio (1) $\left(\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 cc of blood}} \right)$, under certain special conditions of diuresis and high blood urea concentration. That this method is an accurate index of functioning renal tissue has been shown by several considerations, summarized by MacKay (2). In each of three species (rat, rabbit, and dog) the value of the Ratio bears a direct linear relationship to the actual weight of the kidneys. In man, a similar relationship has been demonstrated between the Ratio and body surface, while body surface and kidney weight are in turn in straight line association.

The purpose of this paper is to study in man the relation between the amount of functioning renal tissue (as measured by the Ratio) and the volume of urine per unit of time under conditions designed to induce "minimum" and "maximum" rates of excretion.² MacKay and MacKay (3) have already demonstrated the relation between the blood urea concentration and the amount of functioning renal tissue (similarly measured).

LITERATURE

Under stringent conditions of (a) urea and water administration and (b) withdrawal of dietary fluids Addis (4) found in man a relationship between the amount of kidney tissue and the rates of urine excretion. He suggested that the disturbing influence of extra renal factors on the excretion rates might be diminished by using a "volume quotient" (the quotient obtained by dividing the "maximum" volume per hour by the "minimum" volume per hour) with which to predict kidney mass.

Experimentally, the amount of renal tissue has been altered by subtotal nephrectomy, ligation of renal artery branches, and ligation of

¹ Supported by a grant from the Rockefeller Fluid Research Fund

² It must be emphasized that no true minimum or "maximum" rate of urine excretion is attained. These terms are used throughout this paper for brevity, and signify that the conditions are such as to induce a slow or rapid rate.

kidney poles Bradford (5) found in dogs a "considerable and practically permanent increase in the amount of urine passed" after about two-thirds of the total kidney mass had been excised, after the excision of three-fourths of the kidney mass, the polyuria was more marked Mark (6), also in dogs, found that there was a reduction in the "maximum" rate of urine excretion when only small amounts of kidney remained, while the "minimum" rate of excretion increased Chanutin and Ferris (7) observed the rate of urine excretion when water intake was restricted in rats after kidney pole ligation Table 1 shows the mean values derived from their protocols

TABLE 1

"Minimum" rate of urine excretion in rats from which portions of the kidneys had been removed by pole ligation (After Chanutin and Ferris (7))

Kidney weight per cent of normal	"Concentration test"	
	Cc per 24 hours	Specific gravity
100.0	2.4	1.058
75.6	10.8	1.024
66.6	15.0	1.017
31.2	15.6	1.014

As yet, no data have appeared showing in statistical terms how the rate of urine excretion is related to the mass of kidney tissue Obviously, it would be very useful clinically if one could predict renal mass merely by measuring the volume of urine excreted in a given time It was to determine whether this could be done that the present study was undertaken

METHODS

Five hundred and twenty-four sets of observations, collected in this laboratory during the past 10 years, were analysed by standard statistical methods (8, 9) In each set the data included the (a) Addis Ratio as per cent of normal, (b) maximum rate of urine excretion as induced by water and urea administration, and (c) minimum rate of urine excretion as induced by withdrawing liquids from the diet

Subjects The subjects were, for the most part, ambulatory patients with Bright's disease of varying type, severity, and duration (Some were normal as judged by the Ratio, urinary sediment, and absence of proteinuria) Age varied from 9 to 63 years Body surface varied from 0.83 to 2.50 square meters, with a mean of 1.72 sq. m. The majority were males Many subjects were studied more than once

Diet The diet varied with the type of the disease being treated Most subjects were taking a diet low in salt and adequate in protein and calories

Edema A few subjects were seen when edematous and again in edema-free periods All observations were included in the analysis

Conditions Each subject received a copy of the following printed directions

DIRECTIONS

- 1 After breakfast take no fluids of any sort until next morning
This means no coffee, tea soup milk, etc., as well as no water
- 2 At 9 00 P M that evening void urine and throw it away
Do not void for some hours before 9 00 P M so that you may have no difficulty in emptying the bladder completely
- After 9 00 P M if possible do not void urine again until next morning, but if it is necessary pass the urine directly into the special bottle which is provided
- 3 At 6 00 A M next morning pass urine directly into the special bottle
- 4 At 6 02 A M be immediately after collecting the night urine in the special bottle, drink a large glass of water in which the urea in the box has been dissolved Then during the next half hour slowly drink three other large glasses of water The water may be taken hot if desired
- 5 At 7 A M void and throw away urine Drink two glasses of water slowly
- 6 At 8 A M void and throw away urine Drink two glasses of water slowly
- 7 At 8 50 A M be in the laboratory
- 8 Take no breakfast

After the subject came to the laboratory, his urine was collected and measured for three consecutive hours and blood specimens were withdrawn at the middle of each collection period The rate of urea excretion (mgm in one hour's urine) and the concentration of urea in the blood (mgm in 100 cc) were determined, from this data the Ratio was calculated and expressed as per cent of normal for the subject's body surface (1)

Minimum volume The volume of urine excreted during the restriction of dietary fluids was divided by the elapsed time to obtain the rate of excretion as cubic centimeters per hour This rate was adjusted to the mean body surface of the group (1 72 sq m) before statistical analysis

Specific gravity of the "concentration test" The specific gravity of the urine obtained during restriction of fluids was measured by a hydrometer at room temperature No correction was made for proteinuria

Maximum volume The maximum rate of excretion of urine in any one of the three hour periods during the execution of the Ratio was selected and expressed as cubic centimeters per hour Before statistical analysis, this rate was corrected to a body surface of 1 72 sq m The specific gravity of this urine was not recorded

Volume quotient This variable $\frac{\text{maximum volume}}{\text{minimum volume}}$ was calculated in each of the 524 sets of observations, there was no need to correct it for surface area

Statistical terms A few terms should be explained briefly for those not familiar with statistical methods (8, 9)

a *Correlation coefficient* (r) is a measure of the correlation of two variables when the means of the arrays fall upon a straight line, within the errors of sampling (An array is a row or a column of a correlation table)

b *Correlation ratio* (η) is a measure of the correlation of two variables without regard to the linearity of the means of the arrays. The value of η given here is corrected in each case to allow for the influence of the number of the arrays (18). When $\xi/P E_f$ is greater than 3, η is significantly greater than r , and may be used in place of r ($\xi = \eta^2 - r^2$)

c *Standard deviation* (σ) is a measure of dispersion or variation

d *Regression lines* are lines formed by plotting the means of the arrays. In this study curves have been fitted to them, when possible, by the method of least-squares. In every correlation of two variables, there are two regression lines (one the means of the rows, the other the means of the columns) when they are at right angles, correlation is nil, when they coincide, correlation is unity. It is important to bear in mind that the regression of x on y allows x to be predicted when y is known, the regression of y on x allows y to be predicted when x is known.

RESULTS

The results of the statistical analyses are presented in Table 2, Correlation Tables 3-6, Regression Tables 7-10, and in Figures 1-5

DISCUSSION

General considerations The regressions were curvilinear ($\xi/P E_f > 3$) in the correlations of the Ratio with minimum volume, specific gravity of the concentration test, and volume quotient. The only linear regression ($\xi/P E_f < 3$) was found in the case of the maximum volume, which also showed the highest correlation with the Ratio.

On theoretical grounds one expects that, of two tests of function having the same correlation ratios (η) with the mass of functioning kidney tissue, the test which has the more linear regression with renal weight would be the better test clinically. Thus, the correlation ratio (η) of blood urea concentration with the Addis Ratio is -0.90 ($\xi/P E_f = 4.3$), but a study of the scatter diagram drawn by the MacKays (3), from which η was calculated, shows that the mass of kidney tissue may decrease to as little as 50 per cent of normal before the blood urea changes appreciably in concentration. Despite the high correlation ratio, the fact that the curve of the plotted observations is far from linear signifies that the blood urea concentration is an insensitive test of kidney mass. The MacKays' curve, in fact, approaches the shape of a rectangular hyperbola, any variable which actually formed the latter curve when plotted against kidney weight would plainly be useless as a clinical test, even though the correlation approached unity.

Therefore, the maximum rate of urine excretion, because it happens to have a linear regression and a comparatively high correlation with the Ratio, is the best measure of kidney mass of those variables studied in this paper

TABLE 2

Statistical characteristics of the Addis Ratio (per cent of normal) with minimum volume specific gravity of concentration test maximum volume and volume quotient The Addis Ratio is denoted by x in each instance the other variable by y

Characteristic	Minimum volume	Specific gravity concentration test	Maximum volume	Volume quotient
r	-0.354 ± 0.026	$+0.530 \pm 0.021$	$+0.697 \pm 0.015$	$+0.568 \pm 0.020$
η_{xy} $\xi/PE\xi$	-0.351	$+0.577$ 4.00	$+0.710$ 2.39	$+0.683$ 7.44
η_y $\xi/PE\xi$	-0.399 3.23	$+0.554$ 2.77	$+0.705$ 1.84	
M_x	62.53 ± 0.77	62.53 ± 0.77	62.53 ± 0.77	62.53 ± 0.77
M_y	31.88 ± 0.54	1.0255 ± 0.0002	437.3 ± 5.73	17.95 ± 0.36
σ_x	26.01 ± 0.52	26.01 ± 0.52	26.01 ± 0.52	26.01 ± 0.52
σ_y	18.22 ± 0.38	0.0072 ± 0.0001	194.4 ± 4.05	12.29 ± 0.25
N	524	520	524	524

Regression of Addis Ratio (per cent of normal) on minimum volume

$$x = 78.628 - 0.505y$$

Regression of Addis Ratio (per cent of normal) on specific gravity of concentration test

$$x = 1909y - 1896$$

Regression of Addis Ratio (per cent of normal) on maximum volume

$$x = 0.0933y + 21.728$$

Regression of Addis Ratio (per cent of normal) on volume quotient

$$x = 75.3 + 0.1243y - 60.89e^{-0.1202y}$$

Prediction of the amount of kidney tissue Figure 1 shows in line-chart form the most probable predictions of the amount of functioning kidney tissue (expressed as per cent of the normal Ratio) from a given rate of urine excretion under the conditions outlined above. Each line in the chart represents the calculated regression line of the Ratio on the appropriate variable.

In 50 cases, the Ratio was predicted (by means of Figure 1) from the maximum volume, minimum volume, specific gravity of the concentration test, and volume quotient, the predictions were compared with the actual Ratios and the root mean square deviations (8) of the errors calculated. The deviations were 29.8 for the minimum volume, 19.9 for the maximum volume, and 22.1 for both the volume quotient and the

TABLE 3
Correlation table Addis Ratio (per cent of normal) and minimum volume (cubic centimeters/hour/172 sq. m.)

Minimum volume—cc /hour	Addis Ratio—per cent of normal																								Totals
	0-49	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-99	100-104	105-109	110-114	115-119	
140-149 g			1																						1
130-139 g					1																				1
120-129 g						1																			0
110-119 g																									1
100-109 g												1													0
90-99 g		1			1											1									5
80-89 g																									0
70-79 g				1	1	1	2	1	2	1		1			2	1	1								12
60-69 g	1	1	1		2	1	4	1	1	1		2	1	2											20
50-59 g			2	2	1	2	4	3	2	2	3	3	1		1			3	2	1	2	1			35
40-49 g		1	3	1	1	3	11	3	2	2	3	2		3	5	6	7	5	4	4	1		1		48
30-39 g		3	3	4	2	9	4	5	2	3	8	9	8	6	7	13	15	14	11	7	7	4	2	1	101
20-29 g	1	1	2		2	2	6	3	6	11	10	11	10	14	7	20	10	10	11	5	4	4	2	1	161
10-19 g	1	1	1		2	2	3	1	2	4	4	14	11	11	7	20	10	10	11						129
0-9 g			1						1	1	1	2	2	1	1										10
Totals	3	8	14	11	9	22	34	17	17	26	29	45	33	37	23	45	34	36	31	17	15	11	5	2	524

Minimum volume—cc./hour

TABLE 4
Correlation table Addis Ratio (per cent of normal) and specific gravity of concentration test

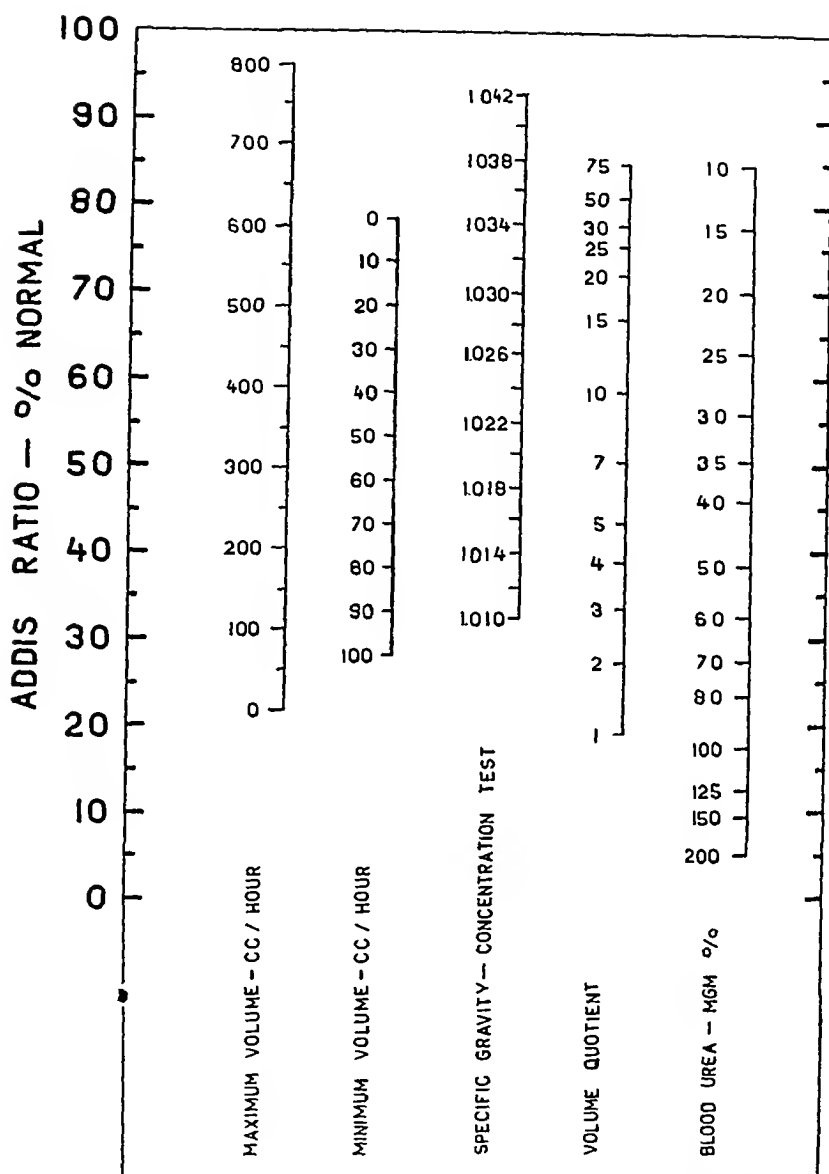
Specific gravity—concentration test	Addis Ratio—per cent of normal																								Totals
	0-49	5-99	10-149	15-199	20-249	25-299	30-349	35-399	40-449	45-499	50-549	55-599	60-649	65-699	70-749	75-799	80-849	85-899	90-949	95-999	100-1049	105-1099	110-1149	115-1199	
1 046-1 047											1	1													1
1 044-1 045											1														1
1 042-1 043												1													2
1 040-1 041																									2
1 038-1 039																									10
1 036-1 037																									13
1 034-1 035																									33
1 032-1 033																									44
1 030-1 031																									56
1 028-1 029																									57
1 026-1 027																									56
1 024-1 025																									52
1 022-1 023																									46
1 020-1 021																									35
1 018-1 019																									28
1 016-1 017																									32
1 014-1 015																									24
1 012-1 013																									24
1 010-1 011																									3
1 008-1 009																									1
1 006-1 007																									
1 004-1 005																									
Totals	3	8	14	11	9	22	34	17	17	26	29	46	33	37	22	44	33	35	30	17	15	11	5	2	520

TABLE 5
Correlation table Addis Ratio (per cent of normal) and maximum volume (cubic centimeters/hour/172 sq m.)

Maximum volume—cc./hour	Addis Ratio—per cent of normal																								Totals
	0-4 9	5-9 9	10-14 9	15-19 9	20-24 9	25-29 9	30-34 9	35-39 9	40-44 9	45-49 9	50-54 9	55-59 9	60-64 9	65-69 9	70-74 9	75-79 9	80-84 9	85-89 9	90-94 9	95-99 9	100-104 9	105-109 9	110-114 9	115-119 9	
960-999															1							1			
920-959																			2						
880-919																	1				1				
840-879								1																	
800-839																			1						
760-799																									
720-759																			1						
680-719																									
640-679																									
600-639																									
560-599																									
520-559																									
480-519																									
440-479																									
400-439																									
360-399																									
320-359																									
280-319																									
240-279																									
200-239																									
160-199																									
120-159																									
80-119																									
40-79																									
0-39																									
Totals	3	8	14	11	9	22	34	17	17	26	29	45	33	37	23	45	34	36	31	17	15	11	5	2	524

TABLE 6
Correlation table *Addis Ratio (per cent of normal) and volume quotient*

Volume quotient	Addis Ratio—per cent of normal																				Totals				
	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-99		100-104	105-109	110-114	115-119
69-71	1												1							1					1
66-68	1																								1
63-65	1																								1
60-62	0																								0
57-59	4																								4
54-56	3											2													3
51-53	3																								3
48-50	3																								3
45-47	6																								6
42-44	10																								10
39-41	9																								9
36-38	13																								13
33-35	24																								24
30-32	27																								27
27-29	40																								40
24-26	39																								39
21-23	43																								43
18-20	51																								51
15-17	48																								48
12-14	51																								51
9-11	58																								58
6-8	57																								57
3-5	31																								31
0-2	524																								524
Totals	3	8	14	11	9	22	34	17	26	29	45	33	37	23	45	34	36	31	17	15	11	5	2		



PLACE A STRAIGHT-EDGE HORIZONTALLY AT THE GIVEN READING
ON THE PROPER SCALE - THE PREDICTED ADDIS RATIO IS FOUND
LATERALLY

FIG 1 LINE CHART FOR PREDICTING ADDIS RATIO FROM MINIMUM VOLUME (Cc/Hr/1 72 Sq M), MAXIMUM VOLUME (Cc/Hr/1 72 Sq M), SPECIFIC GRAVITY OF CONCENTRATION TEST, VOLUME QUOTIENT, AND BLOOD UREA (MGM/100 Cc) (BLOOD UREA LINE CALCULATED FROM DATA OF MACKAY AND MACKAY (3))

specific gravity These results mean roughly that the odds are 2 to 1 in favor of a prediction being within ± 30 per cent³ of the true value when the minimum volume is used, and within ± 20 per cent when any of the other variables are used to estimate renal mass

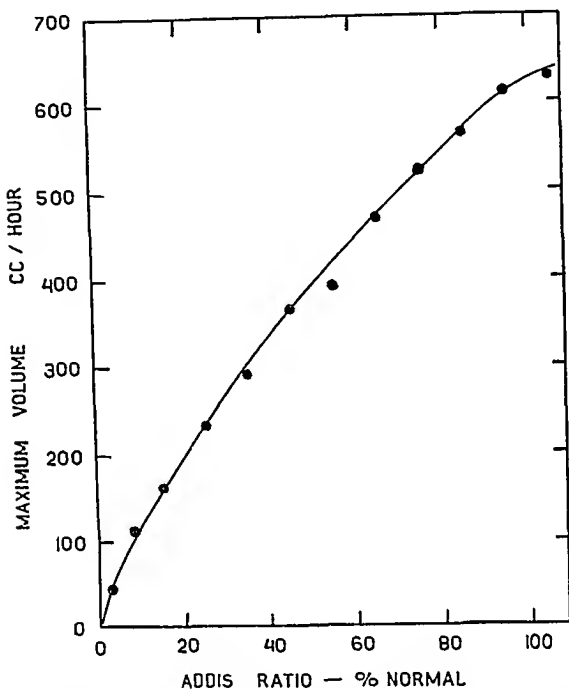


FIG 2 THE REGRESSION LINE OF MAXIMUM VOLUME (CUBIC CENTIMETERS / HOUR / 1.72 SQ M) ON THE ADDIS RATIO (PER CENT OF NORMAL)

Regression lines of the studied variables on the Addis Ratio The regression line of the maximum rate of urine excretion on the Ratio is shown in Figure 2. The means fall quite smoothly along a parabola, which when extended meets the origin. The maximum rate begins to decrease almost as soon as kidney tissue becomes less than normal in amount. The slight lag in response may be an artefact due to the small

³ In per cent of the normal, not of the actual Ratio in any specific instance

number of observations at 105 per cent of normal, or may mean that the stimulus is insufficient to provoke a maximal renal response

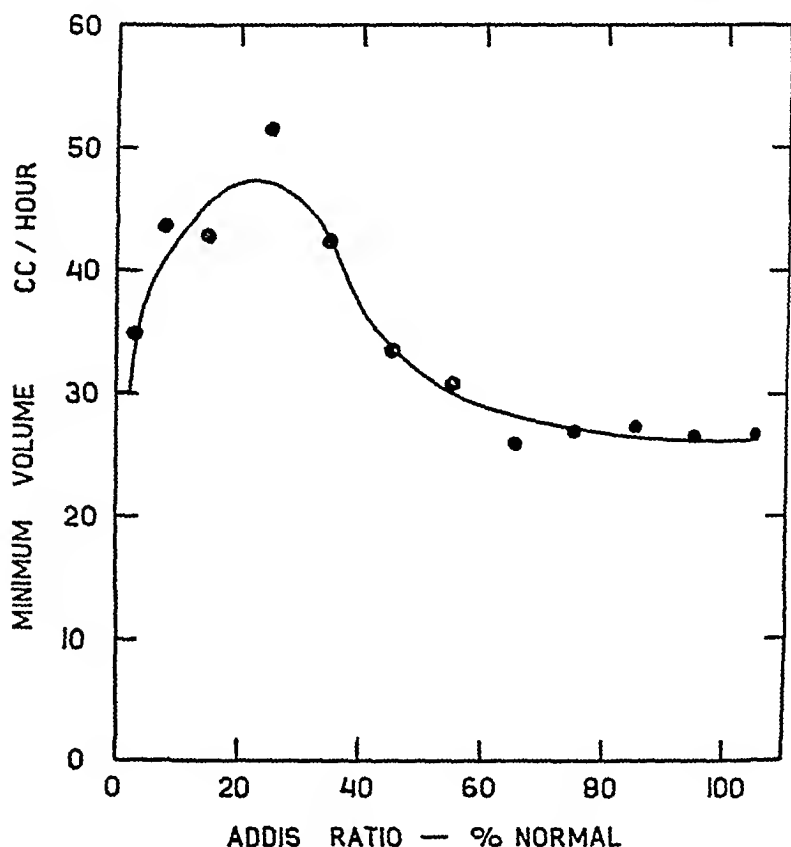


FIG 3 THE REGRESSION LINE OF MINIMUM VOLUME (CUBIC CENTIMETERS /HOUR/1.72 SQ M) ON THE ADDIS RATIO (PER CENT OF NORMAL)

The behavior of the minimum rate of excretion of urine is shown in Figure 3. The curve is not so smooth, and is of a more unusual shape. The capacity of the kidney to produce urine at a slow rate is not involved until about 40 per cent of renal tissue is removed, a polyuria then follows, reaches its height when only 25 per cent of tissue remains, and declines rapidly toward anuria as the mass of kidney disappears.

The regression line of specific gravity of the "concentration test" on the Ratio is given in Figure 4. As kidney substance is removed, the kidney is able to produce urine of high specific gravity until about 20 per cent of the tissue is gone, the urine's specific gravity then rapidly decreases at a rate which does not slacken until only 30 per cent of the original kidney weight remains.

The volume quotient may be looked upon as a measure of the adaptability of the kidney to diametrically opposite stimuli (to excrete urine at a "maximum" and at a "minimum" rate) Figure 5 shows that this

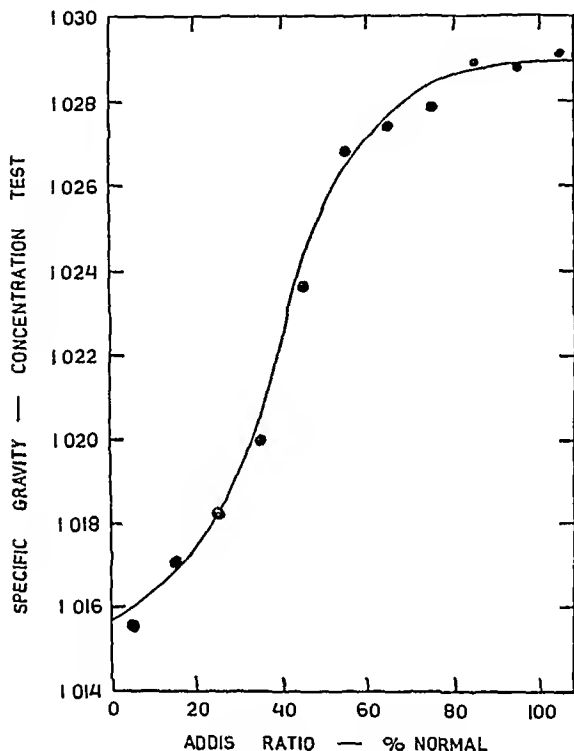


FIG 4 THE REGRESSION LINE OF THE SPECIFIC GRAVITY OF THE CONCENTRATION TEST ON THE ADDIS RATIO (PER CENT OF NORMAL)

adaptability is affected almost as soon as tissue is removed and decreases in a straight line until the kidney is able to excrete urine at only one rate regardless of the stimulus

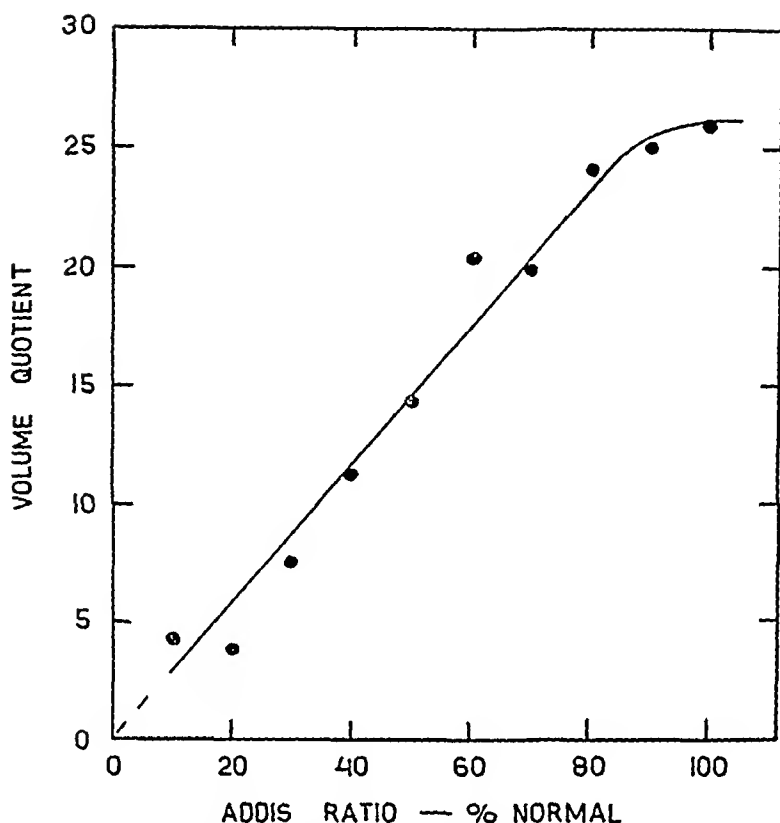


FIG 5 THE REGRESSION LINE OF THE VOLUME QUOTIENT ON THE ADDIS RATIO (PER CENT OF NORMAL)

The data for the four related regression lines are given in Tables 7 to 10

TABLE 7

Regression line of Addis Ratio (per cent of normal) on maximum volume (cubic centimeters/hour/1.72 sq m)

Maximum volume	Addis Ratio	Number of observations
40	23.5	15
120	31.2	34
200	39.9	48
280	47.1	47
360	57.0	75
440	69.7	84
520	74.4	74
600	73.9	64
680	84.8	41
760	88.5	42

TABLE 8

*Regression line of Addis Ratio (per cent of normal) on minimum volume
(cubic centimeters/hour/1.72 sq. m.)*

Minimum volume	Addis Ratio	Number of observations
5	53.5	10
15	72.7	129
25	69.1	161
35	57.8	101
45	50.3	48
55	53.6	35
65	41.7	20
75	42.0	12
85		0
95	38.5	5
105		0
115	22.5	1
125		0
135	27.5	1
145	12.5	1
		<hr/> 524

TABLE 9

Regression line of Addis Ratio (per cent of normal) on specific gravity of the concentration test

Specific gravity	Addis Ratio	Number of observations
1.0055	27.5	1
1.0095	50.8	3
1.0135	32.1	48
1.0175	40.6	60
1.0215	57.3	81
1.0255	64.4	108
1.0295	73.0	113
1.0335	79.9	77
1.0375	81.6	23
1.0415	63.8	4
1.0455	55.0	2
		<hr/> 520

TABLE 10

Regression line of Addis Ratio (per cent of normal) on volume quotient

Volume quotient	Addis Ratio	Number of observations
3	29.7	88
9	52.0	109
15	69.5	99
21	72.9	82
27	75.8	67
33	80.0	37
39	82.0	19
45	87.5	9
51	80.0	6
57	73.5	5
63		0
69	87.5	2
		<hr/> 524

SUMMARY AND CONCLUSIONS

A statistical analysis was attempted in correlating the Addis Ratio (as a measure of the amount of functioning renal tissue) with the rates of excretion of urine under specified conditions designed to induce minimum and maximum rates of excretion

Tests of function are discussed from the view-point of the shape of their regressions with the amount of functioning tissue which they are designed to measure. The shape of the regression is at least as important as the correlation in determining the clinical value of the test

The odds are 2 to 1 that the Ratio may be predicted within a range of ± 20 per cent by means of urine excretion rates

The behavior of the rates of excretion of urine with different amounts of kidney tissue as measured by the Ratio is described. No explanation for the shape of the curves is given

The author desires to thank Dr. Thomas Addis for the kind permission with which his records were used, and for invaluable assistance in the preparation of this paper

BIBLIOGRAPHY

- 1 (a) Addis, T, and Watanabe, C K, J Biol Chem, 1916, xxviii, 251 A Method for the Measurement of the Urea-Excreting Function of the Kidneys
(b) Addis, T, J Urol, 1917, i, 263 The Ratio Between the Urea Content of the Urine and of the Blood After the Administration of Large Quantities of Urea. An Approximate Index of the Quantity of Actively Functioning Kidney Tissue
(c) Addis, T, Arch Int Med, 1922, xxx, 378 Renal Function and the Amount of Functioning Tissue. The Ratio $\frac{\text{Urea in One Hour's Urine}}{\text{Urea in 100 cc of Blood}}$ After Giving Urea and Water
- 2 MacKay, E M, Arch Int Med, 1932, l, 590 Kidney Weight, Body Size and Renal Function
- 3 MacKay, E M, and MacKay, L L, J Clin Invest, 1927, iv, 127 The Relation Between the Blood Urea Concentration and the Amount of Functioning Renal Tissue
- 4 Addis, T, Arch Int Med, 1923, xxxi, 783 The Clinical Significance of Abnormalities in Urine Volumes
- 5 Bradford, J R, (a) Proc Roy Soc London, 1892, li, 25 The Influence of the Kidney on Metabolism (b) J Physiol, 1899, xxiii, 415 The Results Following Partial Nephrectomy and the Influence of the Kidney Upon Metabolism
- 6 Mark, R E, (a) Ztschr f d ges exper Med, 1925, xlv, 1 Ergebnisse partieller Nierenexstirpation am Tiere (b) Ibid, 1928, lix, 601 Untersuchungen uber die Nierenfunktion Ergebnisse partieller Nierenarterienbindung am Hunde
- 7 Chanutin, A, and Ferris, E B, Arch Int Med, 1932, xli, 767 Experimental Renal Insufficiency Produced by Partial Nephrectomy I Control Diet
- 8 Yule, G U An Introduction to the Theory of Statistics Griffin, London, 1929, Ed 9
- 9 Pearl, R Introduction to Medical Biometry and Statistics Saunders, Philadelphia and London, 1930, Ed 2

SALT AND WATER IN THE TREATMENT OF DIABETIC ACIDOSIS

By DAVID M. KYDD

(From the Department of Internal Medicine, Yale University, New Haven)

(Received for publication June 30 1933)

In a previous paper (1) the nature and causes of the bicarbonate deficit and other serum electrolyte changes in diabetic acidosis were discussed from a theoretical standpoint, and disordered electrolyte patterns actually observed were analyzed in the light of this theoretical discussion. It was pointed out that displacement of bicarbonate from combinations with base by ketone acids is a process which can be reversed completely with the contribution from outside sources of nothing except the ability to burn carbohydrate. Base in combination with ketone acids may be considered potential bicarbonate. On the other hand, the loss of base with ketone acids in the urine introduces an irreversible process, because base for the replacement of that sacrificed must come from external sources. Such base deficiencies may become quite extreme, but ordinarily they are small compared with the deficiencies of base combined with chloride. The actual extent of the body chloride losses is hardly evident from the concentrations of this ion found in the serum, because it is masked by contractions of the body fluids. Such a contraction brought about by loss of water in excess of dissolved salts will increase the concentration of chloride more than it will that of bicarbonate, because the body fluids contain more chloride than bicarbonate. In observed cases, the actual reduction of chloride concentration in the serum usually exceeded that of bicarbonate plus undetermined acids (which for convenience was called *S* and represents the actual plus potential bicarbonate mentioned above). If loss of body fluids is taken into consideration it is obvious that the actual loss of chloride must far exceed that indicated by the reduction in concentration. The deficiency of base, of course, exceeds that of either chloride or *S*, because it represents the sum of the two base fractions. In some instances, with *S* well preserved, the reduction of chloride concentration exceeds that of base. This has been interpreted as evidence that the chloride ion, instead of the bicarbonate ion, has yielded base for the neutralization of ketones, a reaction which would seem to be neither useful nor chemically possible. It was pointed out that if equal amounts of base and chloride were excreted and the body fluids were at the same time diminished by a disproportionately large elimination of water, the reduction in concentration of chloride would exceed that of

base, because contraction of fluid volume would increase base concentration more than chloride. It was suggested that this is a more likely explanation of excessive chloride deficits than the previous theory that the chloride ion is excreted with ammonia to provide base for the neutralization of ketones.

The present paper is intended to analyze the clinical and therapeutic implications of these theories in the light of observations made in a series of patients during recovery from diabetic acidosis. The clinical material consists of twenty-five patients on whom studies of blood sugar together with total acid-base equilibrium of the serum were made at frequent intervals by the methods usually employed in this department (2). In certain instances urine was analyzed for total base or chloride or both. The intakes of fluid, sodium, chloride and glucose were usually measured. Records of output are seldom so complete, because urine specimens and vomitus were frequently lost. Of these twenty-five patients, thirteen are discussed below. The remaining cases so closely resembled those reported that the inclusion of additional data does not seem warranted.

In the discussion that follows it is assumed that insulin and carbohydrate were given to all cases with the intention, at least, of re-establishing glucose combustion and thereby eliminating ketosis as rapidly as possible. If this aim is realized, base previously combined with the ketone acids will be released to form bicarbonate. There can be no difference of opinion concerning the objective of therapy in this respect. Differences in the means of securing this objective will be discussed in another connection. The chief concern of the present study is the nature and quantity of fluids and salts which it is advisable to give. For the most part treatment actually employed has been the administration of large amounts of sodium chloride and fluids together with carbohydrate. When patients have been able to take these materials by mouth the sodium chloride has been given chiefly in the form of non-nutritive broth containing 1 per cent of this salt, the carbohydrates as sweetened drinks containing about 10 per cent of sugar. In addition, liberal amounts of water have been permitted. When oral feeding was impossible 0.9 per cent sodium chloride and 5 per cent glucose solutions have been given subcutaneously or intravenously, and occasionally stronger glucose solutions intravenously. The salt solution is intended to restore the volume of the body fluids and to remedy salt depletion. Glucose solution serves two purposes. 1. It provides carbohydrate for combustion and replenishment of glycogen stores. 2. It offers a medium for the parenteral administration of water without salt. Normal salt solution provides sodium in concentration approximately equivalent to that of total base in body fluids. In respiration and insensible perspiration there is a continuous wastage of fluid without any appreciable quantity of salt. Unless the kidneys, then, excrete base in concentration exceeding that of

body fluids, administration of normal saline alone would raise base in the body fluids to an abnormally high level. To obviate this difficulty, in addition to the saline, enough water should be given to provide for losses by extrarenal channels. Glucose solutions answer this purpose because the glucose can be oxidized, leaving only the water.

The analysis of the data presented is complex not only because of the large number of variables with which it is necessary to deal, but also because the serum analyses are concerned with concentrations of substances and give no direct information as to total amounts of fluids and solutes in the body. In a preceding article the average compositions of body fluids in normal subjects and patients with diabetic acidosis were compared (1). In Figure 1, diagrams *A* and *B* represent schematically

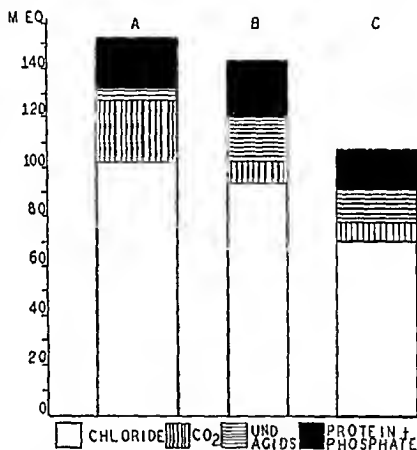


FIG 1. DIAGRAMMATIC REPRESENTATION OF THE CHIEF ELECTROLYTE AND WATER CHANGES THAT OCCUR IN DIABETIC ACIDOSIS

The width of columns *B* and *C* represents roughly the total body fluid volume compared to the normal *A*.

the two conditions. As far as concentrations are concerned they are quite realistic because all values are derived from observed data. The 25 per cent reduction of body fluid in *B* represents little more than a guess, because the actual extent of fluid losses during the development of diabetic acidosis has not been established. Diagram *C* represents the electrolyte concentrations of *B* reduced by dilution to the original fluid volume found in *A*. Thus *C* represents the actual salt losses found in diabetic acidosis. It emphasizes the necessity of having some measure

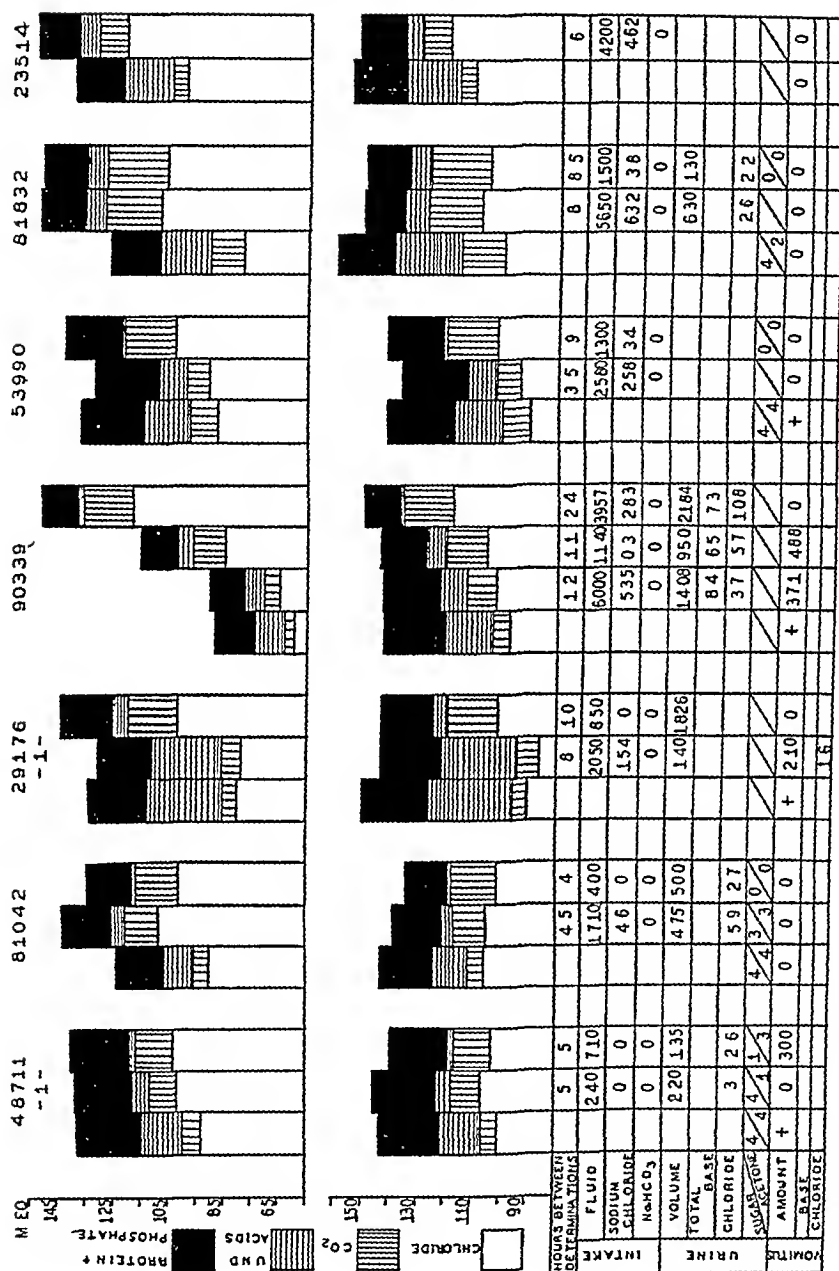


FIG 2

FIGS 2 AND 3 ELECTROLYTE CHANGES DURING RECOVERY FROM DIABETIC ACIDOSIS

The lower diagrams represent the concentrations of electrolytes determined. The upper diagrams represent these data recalculated as described in the text. Total base is not shown but equals the total height of the acid column. Volumes are expressed in cc, electrolytes in mEq. Sugar and acetone in the urine were determined by Benedict's qualitative method and the nitroprusside reaction respectively. The figures 1 to 4 indicate roughly the intensity of the reactions. The specimens of urine and vomitus reported in the first column in each case were produced before treatment was started. The specimens reported in other columns were collected during the periods indicated.

of dehydration in order that electrolyte concentrations determined after various forms of therapy may be adequately compared with the initial concentration. In a previous paper (3) the relation of the serum proteins to hemoconcentration has been discussed. It was pointed out that, except in the presence of shock, the fall of serum proteins from their initially high values probably is associated with, even if it is not exactly proportional to, restitution of body fluid. In the absence of a better and more exact method, the drop in the level of the serum proteins has been used in the succeeding discussion as one measure of body fluid change. Knowing the fluid and salt balances of an individual during a period of time, the changes that occur in the serum electrolyte concentrations afford another measure of body fluid change, if isotonicity of all the body fluids be assumed. It is only by correlating concentrations of electrolytes with changes in body fluid volume that the extent of the original depletion of the salts and their replacement during recovery become apparent.

The analytical data obtained in thirteen of the cases investigated are presented in Figures 2 and 3. The bottom row of diagrams in these figures represents the serum electrolyte concentrations found. Total base is not shown, but equals the total height of the acid column in each case. The top row of diagrams presents the data recalculated to take into account water change. This was accomplished by using the changing serum protein concentrations as an index. For example, in Case 60404, the initial serum protein concentration was 18.9 mEq and the final, 13.9 mEq. Assuming that no protein was lost or gained, the serum volume must have increased 35.9 per cent. All the electrolyte concentrations found at the time of the first determination have been reduced 35.9 per cent in order to eliminate changes in concentration due to differences in the amount of water present. Thus the initial chloride concentration of 98.2 mEq becomes 69.3 mEq. Each determination has been compared to the final one in order that the approximate amounts of each ion added could be ascertained. The amount of salt in the intake and in the urine and vomitus are expressed in milliequivalents. Chloride was given in the form of sodium chloride, so that equal amounts of sodium and chloride were given.

In analyzing the data, not only must the effect of changing body water content on the concentration of the electrolytes be evaluated, but also the results of vomiting and nausea must be ascertained. Unfortunately, vomiting, or at least nausea, is one of the most frequent events during the course of diabetic acidosis. Individuals who are vomiting are obviously losing from the body varying amounts of salt and fluid. They have in the intestinal tract a large volume of fluid which, although isotonic with the body fluids in general, may be of entirely different composition in regard to particular ions. Fluid placed in the gastro-in-

testinal tract eventually becomes isotonic. This is true not only in individuals who are actually vomiting but also in those who are nauseated. When, during recovery, the gastro intestinal symptoms diminish, this fluid is reabsorbed into the mass of body water. These facts explain otherwise incomprehensible alterations in salt concentrations and fluid balances, observed during recovery, which are not related to the amount of salt and fluid immediately taken into the body or excreted. Case 29176-1 furnishes a qualitative example of these changes. In the first eight hours the patient received 2050 cc of fluid with 154 m Eq of sodium chloride. Throughout this time he was vomiting or was nauseated. The water content of the blood was apparently maintained, but base and chloride concentrations diminished, *S* remained constant. In the next 10 hours his nausea disappeared. Although he received only 850 cc of fluid with no salt during this period and passed 1820 cc of urine, there was about a 10 per cent blood dilution with a marked rise of serum chloride. The concentration of base in the serum remained the same, but as the recalculated diagram shows, the total amount of base actually increased. The most reasonable explanation of these changes seems to be that fluid was first poured out into the alimentary canal, and then reabsorbed. This subject forms a part of a general discussion of water balance to be reported by Laviètes, Kydd and Peters (4).

In order to test the effect of varying therapy the individuals have been divided into four groups. 1 The patients who were treated with insulin and carbohydrate alone. 2 The patients who were given fluids in addition. 3 The patients who received varying amounts of salt in addition to the therapy used in 1 and 2. 4 The patients who received bicarbonate as well as salt, fluid, carbohydrate and insulin. It has already been stated that the proportions of insulin and carbohydrate used will not be discussed in this paper.

1 Treatment without salt and fluids

Case 48711-1 was given enough fluid (as carbohydrate drinks) barely to equal the amount of urine that was formed. She received no salt. Initially she had a rather high undetermined acid in the serum, presumably mostly ketones, while *S* was only moderately reduced. With this form of therapy the body fluids evidently became somewhat more contracted, as the total base and chloride rose slightly in the first five hours. The ketones rapidly diminished, but *S* also decreased. Therefore, although its excretion was not measured, it can be inferred that base was excreted. CO_2 rises because of loss of fluid and also because of the base liberated from ketones and not excreted. During the next five hours the patient received slightly more fluid, but still an insufficient amount. *S* is maintained, the undetermined acid fraction is still further reduced and CO_2 augmented by an equal amount. In brief, dehydration

is not overcome and the increase of CO_2 that is observed is due to replacement of the ketones that have been burned and to fluid depletion. The excretion of chloride is very small, 3 m Eq. Although base excretion was not quantitatively determined, the ketones in the urine, evidenced by a heavy nitroprusside reaction, probably carried base with them. Depletion of electrolytes must have continued throughout the period of study in this individual, but its effects on the serum were masked by the simultaneous loss of fluid. The increase of bicarbonate consequently gives a speciously favorable impression of the efficacy of a therapeutic course which was obviously unsatisfactory if its aim was the restoration of a normal internal environment.

2 Treatment with fluids alone (Cases 81042, 29176-1)

There are no cases that received fluids entirely without salt. The cases listed in this group received less salt than they excreted. Case 81042 was given 2120 cc of fluid and only 46 m Eq of chloride during a period of eighty-two hours in which she excreted 975 cc of urine containing 86 m Eq of chloride. No figures for the excretion of base are available. The serum proteins drop from 8.09 per cent to 6.92 per cent, evidencing adequate hemodilution. The CO_2 concentration rises because of displacement of ketones. Total base and chloride fall, not only because dilution takes place, but because chloride at least was lost in the urine. Base falls farther than chloride because, since its concentration is higher in the serum, it is more affected by dilution. This phenomenon is discussed in detail elsewhere. This therapy causes dilution of the electrolytes. Any augmentation of CO_2 which occurs is referable only to the base liberated by burning ketones. Again, consideration of bicarbonate alone gives an erroneous impression of the results of therapy, and especially of its effects on base.

3 Treatment with varying amounts of salt and fluid (Cases 90339, 53990, 81832, 23514, 60404, 71954-2)

This has been the accepted mode of treatment and most of the cases reported fall within this group. The diagrams of individuals exhibit responses of great variability because of differences in initial electrolyte and fluid depletion, the proportions and amounts of salt and fluid given, the amount of vomiting and nausea and the quantities of fluid and salt excreted.

Case 90339 had in the serum an initial chloride concentration of 97 m Eq with a total base of 142.8 m Eq. During the first twenty-two and three-quarter hours she received (4792 cc) more fluid than she excreted. Her salt intake was 548.3 m Eq of sodium, 28.8 m Eq of potassium and 535.2 m Eq of chloride. She excreted 90.7 m Eq of sodium, 51.6 m Eq of potassium and 93.7 m Eq of chloride. With this

therapy the serum chloride concentration rose to 106 m Eq. Serum CO_2 rose because bicarbonate replaced the ketones that were burned and S also increased slightly. This is not easily apparent from the concentrations observed, but becomes so when the extent of original fluid loss, which is represented in the diagram, is considered. During the ensuing twenty four hours she received 1873 cc of fluid more than she excreted, together with 286 m Eq of sodium and 283 m Eq of chloride, of which she excreted 52 and 107.8 m Eq respectively. At the end of this time her chloride had risen to 118 m Eq notwithstanding further dilution. Base was now normal, 150.2 m Eq. CO_2 , however, had only risen 3 m Eq and was still very low, 17.8 m Eq. Despite the distorted electrolyte pattern observed at the end of the study, the patient, aside from exhaustion, had felt entirely well for more than twenty four hours.

Case 53990 on admission was vomiting profusely and was markedly dehydrated. Vomiting and nausea ceased soon after therapy was instituted. During the first three and one half hours he received 2580 cc of fluid with 258 m Eq of sodium chloride. Although the ketones diminished, CO_2 fell, presumably because of the continued heavy excretion of the former together with a certain amount of base. In the recalculated diagram it is observed that S fell 8 m Eq, whereas total base fell 4.8 m Eq and chloride rose 3.4 m Eq. The amount of sodium chloride added to the serum was apparently insufficient to replace the base and ketones lost. During the next nine hours very little sodium chloride was given, 34 m Eq. Despite slight retention of fluid, chloride and total base rose rapidly in a parallel fashion. CO_2 was increased by further elimination of ketones. Because of the low intake during this period these changes can be interpreted only as the result of absorption of fluid from the gastro intestinal tract.

Case 81832 was also given large amounts of salt and fluid. In this case salt depletion was not extreme. The effect of the treatment was to cause dilution of the serum with a marked increase of base and chloride. If the observed changes in the electrolytes in this case are recalculated with consideration of the dilution indicated by the diminishing serum proteins, it is found that equal amounts of sodium and chloride have been retained. The increase in CO_2 has been almost entirely at the expense of ketones. In Case 71954-2 the initial serum chloride is high, but base is very low, as are the undetermined acids. In the course of thirteen and one quarter hours he received 4010 cc of fluid more than he excreted and retained 505 m Eq of chloride. The large amount of fluid given caused extreme dilution of the serum. Base slowly rises. Chloride, after an initial rise, returns almost to its original concentration. Unfortunately, no base excretion figures are available, but, as S slowly increases, chloride must have been excreted in equal or greater amounts than base. Because chloride concentration in serum is greatly less than base an excretion of

equal amounts of each will cause a relative increase in serum base concentration

The administration of both sodium chloride and water permits restoration of base and body fluids. Hyperchloremia often results and bicarbonate may remain low. However, these abnormalities of the electrolyte pattern do not seem to have any deleterious effects as far as one can judge from objective and subjective clinical signs.

4 *Treatment with fluid, sodium chloride and sodium bicarbonate* (Cases 29176-2, 71954-3, 85138, 29176-3)

Sodium bicarbonate was given to four cases, but no conclusive results were obtained. Case 29176 during the first four hours received 119 m Eq of sodium bicarbonate, 285 m Eq of sodium chloride and 2650 cc of fluid. Serum base rose from 141 to 151 m Eq, chloride from 93.2 to 95.6 m Eq. *S* rose from 23.8 to 36.3 m Eq, but CO_2 rose only from 6.7 to 12.0 m Eq, indicating a very considerable increase in the undetermined acids. During the next four hours no more bicarbonate was given. Further dilution took place. Base concentration fell, as did *S*, but the undetermined acids were greatly reduced, so that CO_2 rose. The chloride concentration rose to normal, 101.8 m Eq. These changes become more comprehensible in the upper figures, where body fluid changes are taken into consideration. It is then apparent that during the first four hours CO_2 increases because of an accession of total base. During the second four hours CO_2 replaces the ketones and total base and chloride are added in about equal quantities. The reason for the increase in ketone acids after bicarbonate administration is obscure. It is known that bicarbonate will increase the excretion of ketones and in this case qualitative ketones persisted in the urine for a longer period than was expected. It is conceivable that the ketones came from the cells in response to the addition of base to the extracellular fluids.

In Case 71954-3, 107 m Eq of sodium bicarbonate, 247 m Eq of sodium chloride and 3800 cc of fluid were given in eight hours. He developed a hyperchloremia and the concentration of CO_2 rose only by virtue of the base liberated by oxidation of ketones. This patient was vomiting on admission and nausea continued for more than three hours. That he then absorbed from his stomach considerable free hydrochloric acid, part of which had been neutralized by the small amount of bicarbonate given, would provide a plausible explanation of the data. In Case 29176-3 much the same phenomena occur. In the last period of four hours 71 m Eq of sodium bicarbonate without any chloride were given. During this four hours chloride and base rose. The concentration of CO_2 increased from 10.8 to 20.5 m Eq, but the concentration of undetermined acids fell from 20.7 to 6.6 m Eq, so that *S* actually diminished. The sodium bicarbonate given was more than neutralized.

by the contents of the gastro intestinal tract while the individual was nauseated. The vomitus secured for analysis contained 33.6 mEq of total base and 40.4 mEq of chloride. Case 85138, in four hours, received 1810 cc of fluid, 155 mEq of sodium chloride and 95 mEq of sodium bicarbonate. *S* remained constant. The recalculated diagram shows that equivalent amounts of total base and chloride were added to the serum. Undoubtedly, sodium bicarbonate was not given in sufficiently large amounts to produce a demonstrable specific effect upon serum bicarbonate.

DISCUSSION

The cases are complicated by differences in initial salt and water depletion, the occurrence of vomiting or nausea, and variation in renal function, as well as by the type of therapy used. However, certain inferences can be drawn from analysis of the data. Treatment with carbohydrate and insulin alone is unsatisfactory. It does not supply the elements that have been wasted by the body. Dissipation of saltless water by means of insensible perspiration and of salt and water through renal activity and vomiting continue. These processes lead to a concentration of all the solutes in the interstitial fluids. If the initial loss of salt is small, the concentrations of serum electrolytes may appear surprisingly normal unless the degree of body fluid loss is considered. Treatment with fluid without salt is also unsatisfactory. Dilution occurs, making the salt depletion even more apparent. In either instance, changes in the concentration of CO_2 do not indicate any essential improvement in the patient's condition.

The CO_2 may rise further when no fluid is given because the body fluids become more concentrated. Nevertheless, administration of fluid at least has the advantage of combating, to some extent, dehydration. In both cases increases of CO_2 are derived only from base liberated by combustion of ketones, while the actual base content of the body must suffer loss through urine and vomitus.

Since sodium, the major basic ion of intestinal fluids, cannot enter cells, the rising osmolar concentration of extracellular fluids that occurs when no salt and no fluid is given, must force the cells to give up water. Atchley, Loeb, Richards, Benedict and Driscoll (5) showed that during development of the dehydration of diabetic acidosis intracellular as well as extracellular fluid is lost, so that further concentration is even more undesirable. If fluid without salt be given, the osmotic concentration of the extracellular fluids would diminish, forcing the cells to imbibe water or perhaps to lose base. Carried far enough this would lead to complete disruption of the internal environment.

The importance of replacing the salt and fluid lost from the body has already been discussed in a previous article (3) in relation to shock and to the continuance of the stupor and other clinical symptoms. The rapid

recovery of Case 82529, without any change in the concentration of serum bicarbonate, under the influence of parenteral administration of normal sodium chloride solution, was cited

Three cases have been cited in which salt and fluid in varying amounts produced electrolyte patterns that were distorted and yet were more in keeping with a normal internal environment than most of the patterns resulting from either of the other forms of therapy discussed. Hyperchloremia with a continuously low CO_2 concentration is frequently observed. This is shown strikingly in Case 90339. After forty-seven hours the serum of the patient had a chloride concentration of 118 m Eq and a CO_2 concentration of only 17.8 m Eq. The concentration of her serum base was, however, normal, 150.6 m Eq. That this distortion is caused by a dilution of the serum with sodium chloride solution has already been pointed out. In diarrhea of infants, in which base is lost in excess of chloride, Hartmann (6) has claimed that treatment with sodium chloride and water alone is unsatisfactory because it gives rise to hyperchloremia and still further depresses bicarbonate. However, the depression of bicarbonate caused by sodium chloride is not due to displacement of one acid by the other, but to dilution of the bicarbonate by chloride solution. Hoag and Marples (7) have further pointed out that hyperchloremia occurs only when the amounts of chloride solution given are too small to ensure an adequate flow of urine. The effect of renal activity is shown by Case 71954-3. During the first eight hours the serum chloride concentration rose from 98.5 to 106.6 m Eq, although the base concentration remained constant at 140 m Eq. During this period the excretion of total base, 22.2 m Eq, almost equalled the excretion of chloride, 24.9 m Eq. Although his CO_2 concentration at this time was only 14.3 m Eq the patient was entirely asymptomatic. During the next sixteen hours 184.1 m Eq of chloride were excreted with only 31.1 m Eq of base. Unfortunately the electrolyte concentrations were not determined at the end of this time.

The presence of an extremely small chloride excretion at the time when hyperchloremia exists in the serum is also illustrated by Case 60404. Serum chloride concentration rose from 95.2 to 105 m Eq in five hours. During this period 260 m Eq of chloride were administered but only 8 m Eq were found in 556 cc of urine. Extraordinary serum chloride concentrations are seen after clinical recovery. In Case 23514 the final chloride concentration was 120.7 m Eq. Hartmann and Smyth (8) found that in pyloric obstruction, when chloride became depleted, patients, in spite of the retention of abnormally large amounts of CO_2 , excreted acid urine and thus maintained a normal concentration of base in the body fluids. In recovery from diabetic acidosis, under administration of sodium chloride, the reverse obtains. Until a normal base concentration is restored, the excretion of chloride remains low, even when serum chloride has been driven to an exceedingly high level.

Despite the low CO_2 concentration and the existence of hyperchloremia, the patients are entirely free of symptoms, provided that fluid and base requirements have been satisfied. In no case has the actual minute volume of respiration been determined, but in all cases which have progressed to this point, all visible hyperpnea has disappeared, although the bicarbonate concentration may be still as low as that observed in patients who, during the development of acidosis, suffered from extreme air hunger. The maintenance of a low alveolar CO_2 tension with constant CO_2 production must, undoubtedly, necessitate increased breathing. However, even a 50 per cent reduction of CO_2 tension, once established, requires only 100 per cent overventilation, a degree of hyperventilation that is hardly noticeable. Furthermore, during recovery the CO_2 tension is rising. It would seem that the restitution of volume and ionic concentration of the body fluids is of prime importance, whereas, the replacement of individual anions to recreate the normal pattern is of secondary importance. This rearrangement may not occur until long after the disappearance of all untoward symptoms and reactions. Field (9) and others have asserted that the bicarbonate deficit itself inhibits glucose utilization and have advanced this as a reason for administration of bicarbonate. In physiologic or clinical studies no basis for such an assertion is to be found. It has been demonstrated that administration of acidifying salts does not impair oxidation of sugar (10). In fact, Haldane (11) and others claim that it has an accelerating action on such oxidations. In other diseases with comparable acidosis, for example, nephritis, carbohydrate combustion seems not to be affected. Furthermore, it has been observed in the present series that utilization of sugar during recovery from diabetic acidosis may advance to the appearance of hypoglycemia while bicarbonate is still greatly depleted. In patient 79156 the blood sugar fell in successive observations from 427 to 277 to 117 mgm per cent with corresponding bicarbonate values of 3.2, 6.4 and 9.7 mEq. During this period of 6.5 hours he received more than 80 grams of sugar. There can be no doubt that he was burning carbohydrate rapidly at the time of the second and third blood examinations, although the bicarbonate was still at levels usually associated with coma.

Hartmann and Darrow (12) have presented a more logical argument for bicarbonate administration. It is evident from the serum electrolyte patterns at the height of acidosis that the concentration of S is, in many cases, diminished. The actual quantity of S must, of course, be even more depleted by loss of body fluids. That hyperchloremia develops when sodium chloride solution is given in the attempt to replace this deficit has already been demonstrated. In order, then, to restore the normal electrolyte pattern as speedily as possible, administration of bicarbonate would seem advisable. However, dilution of the extra-

cellular fluids with sodium bicarbonate solution alone would cause greater distortion of the pattern than would dilution with sodium chloride, because of the difference in concentration of chloride and bicarbonate found in normal serum. Hence, a combination of the two salts should be given. It has already been stated that the amounts of sodium bicarbonate that were given to patients in this series were too small to permit any adequate deductions.

To be effective, large amounts of this salt would have to be given. Administering the salt by mouth is usually impossible because of the frequent occurrence of nausea and vomiting. This necessitates discontinuing all oral feeding and resorting entirely to parenteral administration of fluid, carbohydrate, and salts. The time required to prepare a solution of sodium bicarbonate for intravenous use precludes its use until the patient should be well on the way towards recovery, if large amounts of fluid and sodium chloride are given. Cunningham and Darrow (13) have proposed a solution made by mixing suitable proportions of sodium bicarbonate and hydrochloric acid. This solution, however, contains but 30 m Eq of sodium bicarbonate per liter, an amount too small to evoke an adequate effect. Hartmann and Senn (14) have proposed using normal sodium lactate solution in amounts varied according to the estimated needs of the individual. This solution provides a means of giving an adequate amount of base to combine eventually with CO_2 . However, the solution also is not immediately available because it must be given intravenously and, therefore, cannot be made in advance if non-specific reactions are to be avoided.

Although it may be desirable to give sodium bicarbonate or a substitute, this salt will not furnish all the requirements of the depleted body fluids. Chloride has been lost in large amounts. The administration of large amounts of sodium chloride as early in the course of treatment as possible is quite essential in accelerating recovery and should not be delayed until a suitable bicarbonate solution can be prepared.

SUMMARY

The effect, on the recovery from diabetic acidosis, of administering varying amounts of fluid, sodium chloride, and sodium bicarbonate, has been discussed. Treatment with carbohydrate and insulin alone, or with the addition of water only is unsatisfactory. The giving of sodium chloride leads to rapid recovery, even though hyperchloremia develop. The individual appears to retain chloride in high concentration until the base concentration approaches normal. Chloride is then excreted in excess of base. Clinical improvement parallels the restoration of body fluid and total ionic concentrations rather than the replacement of individual ions.

BIBLIOGRAPHY

- 1 Peters, J P, Kydd, D M, Eisenman, A J, and Hald, P M, *J Clin Invest*, 1933, xii, 377 The Nature of Diabetic Acidosis
- 2 Peters, J P, Wakeman A M, Eisenman, A J and Lee, C, *J Clin Invest*, 1929, vi, 517 Total Acid Base Equilibrium of Plasma in Health and Disease X The Acidosis of Nephritis
- 3 Peters, J P, Kydd, D M, and Eisenman A J, *J Clin Invest*, 1933, xii, 355 Serum Proteins in Diabetic Acidosis
- 4 Lavietes, P H, Kydd, D M and Peters J P, Unpublished studies
- 5 Atchley, D W, Loeb R F, Richards D W, Jr, Benedict E M and Driscoll, M E *J Clin Invest* 1933, xii, 297 On Diabetic Acidosis A Detailed Study of Electrolyte Balances Following the Withdrawal and Reestablishment of Insulin Therapy
- 6 Hartmann A F, *Am J Dis Child* 1928 xxxv 557 Chemical Changes Occurring in the Body as the Result of Certain Diseases I The Effects of Diarrhea, Vomiting, Hydration and Oliguria on the Acid Base Balance of the Plasma of Infants with Mastoiditis
- 7 Hoag L A and Marples E *Proc Soc Exper Biol and Med*, 1929, xxvi 374 Treatment of Dehydration of Diarrhea with Parenteral Fluids 1 Effect on Acid Base States of Blood
- 8 Hartmann A F and Smyth F S *Am J Dis Child*, 1926 xxxii, 1 Chemical Changes in the Body Occurring as the Result of Vomiting
- 9 Field, H Jr, Personal communication cited by Peters and Van Slyke, *Quantitative Clinical Chemistry 1 Interpretations* Williams and Wilkins Company, Baltimore 1931 p 170
- 10 Gilchrist, M L *Arch Dis Child* 1932 vu, 69 The Influence of Acidosis on Carbohydrate Metabolism
- 11 Haldane, J B S, *Lancet* 1924 i 537 Experimental and Therapeutic Alterations of Human Tissue Alkalinity
- 12 Hartmann A F and Darrow D C *J Clin Invest*, 1928 vi 257 The Composition of the Plasma in Severe Diabetic Acidosis and the Changes Taking Place during Recovery
- 13 Cunningham R D M, and Darrow, D C, *Am J Dis Child* 1931, xli 1347 Preparation of a Solution of Sodium Bicarbonate and Sodium Chloride for Hypodermoclysis
- 14 Hartmann A F and Senn J E *J Clin Invest* 1932, xli, 337 Studies in the Metabolism of Sodium *r* Lactate 11 Response of Human Subjects with Acidosis to the Intravenous Injection of Sodium *r* Lactate

INDEX TO VOLUME XII

- Agranulocytosis treatment with adenine sulphate Paul Reznikoff 45
- Albright Fuller 974
- Allison C L See Johnston C G
- Alvarez Walter C See Osterberg Arnold E and Vanzant Frances R
- American Society for Clinical Investigation Proceedings of the Twenty fifth Annual Meeting of held in Washington D C May 8 1933 963
- Amoss Harold L and Craven Erle B Jr Serum treatment of hemolytic streptococcus pneumonia 885
- Andrus E Cowles 975
- Anemia of infancy from maternal iron deficiency Maurice B Strauss 345
- Anemia pernicious glycolysis in the blood S Milton Goldhamer 583
- Anemia pernicious in pellagra Tom Douglas Spies and Warren Payne 229
- Anemia pernicious pancreatic enzymes in O M Helmer Paul J Fouts and L G Zerfas 519
- Angina experimental Plaut Vincent s in dogs Helene Wallace E W Wallace and O H Robertson 909
- Arthritis See rheumatoid arthritis
- Arthritis agglutination reaction in Edith E Nicholls and Wendell J Stansby 505
- Arthritis sedimentation rate of red blood cells Theodore W Oppel and Chester S Keefer 291
- Ask Upmark Eric 986
- Atchley Dana W Loeb Robert F Richards Dickinson W Jr Benedict Ethel M and Driscoll Mary E On diabetic acidosis a detailed study of electrolyte balances following the withdrawal and reestablishment of insulin therapy 297
- Aub Joseph C See Meyer Ovid O
- Ayman David See Proger Samuel H
- Baker, Benjamin M Jr 975
- Baker James P 992
- Barach Alvan L., 975
- Barker Paul S 969, 993
- Barnett C W, The unavoidable error in the differential count of the leukocytes of the blood, 77
- Bauer Walter, 967
- Bedell Caroline C 975
- Beecher Henry K. The measured effect of laparotomy on the respiration, 639
- Effect of laparotomy on lung volume. Demonstration of a new type of pulmonary collapse, 651
- Bellet Samuel 970
- Benedict Ethel M See Atchley, Dana W
- Bernstein, Clarence 969
- Binns Dorothy See Muntwyler Edward
- Blake Francis G 969
- Bland Edward F 993
- Bliss Eleanor A., 965
- Bliss Eleanor A See Long, Perrin H.
- Blood colloid osmotic pressure formula and nomogram for estimation of, Herbert S Wells John B Youmans and David G Miller Jr, 1103
- Blood flow to the lower extremities effects of alternate suction and pressure on, Eugene M Landis and John H Gibbon Jr 925
- Blood oxygenation of concentrated versus normal George B Ray, C I Thomas and J E Strong 1051
- Blood plasma lipids in epilepsy I Irvine McQuarrie W R Bloor Clara Husted and H A Patterson 247
- Blood plasma lipids in epilepsy II Irvine McQuarrie Clara Husted and W R. Bloor 255
- Blood plasma lipoids permeability of capillaries to, Evelyn B Man and John P Peters 1031
- Blood plasma protein and colloid osmotic pressure See edema
- Blood pressure in the brachial arteries inequality of Horace M Korns and P H Guinand

- Blood proteins, subcutaneous lymph in, A A Weech, E Goettsch and E B Reeves, 1021
- Blood serum proteins in diabetic acidosis John P Peters, David M Kydd and Anna J Eisenman, 355
- Blood The unavoidable error in the differential count C W Barnett, 77
- Bloor, W R See McQuarrie, Irvine
- Blumgart, Herrman L, 975
- Bohning, Anne, 973
- Boller, Charles See Hurtado, Alberto
- Bowen, Byron D, 976
- Bowie, Morris A See Klumpp, Theodore G
- Brown, George E, 977
- Bruger, Maurice, 985
- Bulger, Harold A and Gausmann, Florence, Magnesium metabolism in hyperparathyroidism, 1135
- Burwell, C Sidney, 977
- Callahan, William See Smyth, Francis Scott
- Capillaries, permeability of, to plasma proteins Evelyn B Man and John P Peters, 1031
- Capillary wall, effects of temperature and tissue pressure on the movement of fluid through, Eugene M Landis and John H Gibbon, Jr, 105
- Cardiac failure, cisternal pressure and orthopnea William Groce Harrison, Jr, 1075
- Cardiac output, methods for determining, Arthur Grollman, Ben Friedman, Gurney Clark and T R Harrison, 751
- Carpenter, Harriet M, 965
- Carpenter, Harriet M See Long, Per-rin H
- Chang, Hsiao-Ch'ien, Gastric secretion in fever and infectious diseases, 155
- Chasis, Herbert, Jolliffe, Norman and Smith, Homer W, The action of phlorizin on the excretion of glucose, xylose, sucrose, creatinine and urea by man, 1083
- Christie, Ronald V, 974
- Churchill, Edward D, 978
- Clark, Gurney, 981
- Clark, Gurney See Grollman, Arthur
- Cobb, Stanley, 967
- Coggeshall, Lowell T See Robertson, Oswald H and Terrell, Edward E
- Collins, L H, Jr, 988
- Collins, Leon H, Jr See Starr, Isaac, Jr
- Cope, Cuthbert L, Studies of urea excretion VIII The effects on the urea clearance of changes in protein and salt contents of the diet, 567
- Craig, Winchell McK, Horton, Bayard T and Sheard, Charles, Thermal changes in peripheral vascular disease during sympathetic ganglionectomy under general anesthesia, 573
- Craven, Erle B See Amoss, Harold L
- Cullen, Glenn E See Wilkins, Walter E
- Curran, Jean A, 979
- Curtis, George M, 963
- Darrow, Daniel C See Yannet, Herman
- Dawson, M H, 978
- DeGraff, Arthur C, 979
- Derick, Clifford L, 979
- DeWolf, H F, 991
- Diabetes, influence of, on the development of antibacterial properties in the blood Russell Richardson, 1143
- Diabetic acidosis Electrolyte balances following insulin therapy Dana W Atchley, Robert F Loeb, Dickinson W Richards, Jr, Ethel M Benedict and Mary E Driscoll, 297
- Diabetic acidosis, nature of, John P Peters, David M Kydd, Anna J Eisenman and Pauline M Hald, 377
- Diabetic acidosis, salt and water in the treatment of, David M Kydd, 1169
- Diabetic acidosis, serum proteins in, John P Peters, David M Kydd and Anna J Eisenman, 355
- Dickie, Lois F N, 990
- Dickie, Lois F N See Thompson, Wil-ard Owen
- Digitalis, effect on venous pressure David A Rytdand, 847
- Diphtheritic intoxication, experimental I Blood sugar, lactic acid, nonprotein and amino-acid nitrogen Herman Yannet and Daniel C Darrow, 767
- Diphtheritic intoxication, experimental II Hepatic glycogenesis and glycogen concentration of cardiac and skeletal

- muscle Herman Yannet and Daniel C Darrow, 779
- Diphtheritic intoxication, experimental, III Respiratory quotients and metabolic rate. Herman Yannet and Walter Goldfarb 787
- Diuretics See renal function
- Donal, J S, 988
- Donley, Dorothy 965
- Driscoll Mary E See Atchley, Dana W
- Edema in dogs, effects of serum transfusion. A A Weech E Goettsch and E. B Reeves 217
- Edema, nephrotic mineral metabolism. William S Hoffman and Wilber E Post, 613
- Edema plasma protein and colloid osmotic pressure in, Edward Muntwyler, Charles T Way Dorothy Binns and Victor C Myers 495
- Edema, relation between plasma protein and specific gravity and edema in dogs A A. Weech C E Snelling and E Goettsch 193
- Eisenman, Anna J See Peters John P
- Electrolytes in human tissue. III Hearts, normal and in congestive failure Walter E. Wilkins and Glenn E Cullen, 1063
- Ernstene, A Carlton 980
- Ethridge Clayton 993
- Evans Frank A 972
- Fever in infectious disease gastric secretion in Hsiao Chien Chang 155
- Finesinger Jacob E 967
- Finland Maxwell 972
- Fisher, Luther C 968
- Fouts, Paul J See Helmer, O M
- Fray, Walter W See Hurtado Alberto
- Fremont Smith Frank 980
- Friedenwald Jonas S 969
- Friedman, Ben 981
- Friedman, Ben. See Grollman, Arthur
- Gallbladder bile, anion-cation content C. G Johnston I S Ravdin C Riegel and C L. Allisoo 67
- Gamble, C J, 988
- Garner, R. L. 991
- Gastric anacidity blood in W Scott Poland, 599
- Gastric pepsin I Methods Arnold E Osterberg Frances R. Vanzant and Walter C. Alvarez, 551
- Gastric pepsin II In duodenal ulcer Frances R. Vanzant, Arnold E. Osterberg Walter C. Alvarez and Andrew B Rivers, 557
- Gastric secretion Theodore G Klumpp and Morris A Bowie, 1
- Gastric secretion in fever and infectious diseases Hsiao Chien Chang 155
- Gastritis experimental, attempts to produce, Francis D W Lukens, 181
- Gates, L R. 990
- Gausmann Florence See Bulger, Harold A
- Gibboo, John H Jr, 983
- Gibbon, John H Jr See Landis Eugene M
- Gibbs E L 984
- Gibbs, F A, 984
- Gilligan, Dorothy Rourke 975
- Goettsch, E See Weech A A
- Goldfarb Walter See Yannet Herman
- Goldhamer S Milton, Glycolysis in the blood of patients with pernicious anemia 583
- Gordon Burgess, 981
- Grabfield G P, 964
- Griffith, Fred R Jr 976
- Grollman Arthur 981
- Grollman Arthur Friedman, Ben, Clark Gurney and Harrison, T R, Studies in congestive heart failure. XXIII A critical study of methods for determining the cardiac output in patients with cardiac disease, 751
- Grout Gerald C, 993
- Gunand, P H See Korns Horace M
- Hald Pauline M. See Peters John P
- Halsted J A. See Hayman J M Jr
- Hamilton, Burtoo E 972
- Harrison T R. 981
- Harrison T R See Grollman, Arthur
- Harrison, William Groce, Jr, The internal pressure in congestive heart failure and its bearing on orthopnea 1075
- Hayman J M, Jr Halsted J A. and Seyler L. E, A comparison creatinine and urea of kidney function, 861

- Hayman, J M, Jr and Johnston, S M, Experiments on the relation of creatinine and urea clearance tests of kidney function and the number of glomeruli in the human kidney obtained at autopsy, 877
- Heart, basal work and output Isaac Starr, Jr, Leon H Collins, Jr and Francis Clark Wood, 13
- Heat cramps John H Talbott and Jost Michelsen, 533
- Helmer, O M, Fouts, Paul J and Zerfas, L G, Gastro-intestinal studies II Pancreatic enzymes in pernicious anemia, 519
- Herrmann, George R, 982
- Herrmann, Louis G, 982
- Hill, Ian G W, 993
- Hirschfelder, Arthur D, 982
- Hitchcock, Charles H and Swift, Homer F, The agglutinating properties of exudates from patients with rheumatic fever, 673
- Hoffman, William S and Post, Wilber E, The influence of mineral metabolism upon nephrotic edema, 613
- Horton, Bayard T See Craig, Winchell McK.
- Humphreys, Katharine, 985
- Humphreys, Katharine See Powers, John P
- Hunt, Hazel M, 987
- Hurtado, Alberto and Boller, Charles, Studies of total pulmonary capacity and its subdivisions I Normal, absolute, and relative values, 793
- Hurtado, Alberto and Fray, Walter W, Studies of total pulmonary capacity and its subdivisions II Correlation with physical and radiological measurements, 807
- Studies of total pulmonary capacity and its subdivisions III Changes with body posture, 825
- Hurtado, Alberto, Fray, Walter W and McCann, Wm S, Studies of total pulmonary capacity and its subdivisions IV Preliminary observations on cases of pulmonary emphysema and of pneumoconiosis, 833
- Husted, Clara See McQuarrie, Irvine
- Hyperparathyroidism, magnesium metabolism in, Harold A Bulger and Florence Gausmann, 1135
- Hypertension, arteriolar, hyperventilation in, Samuel H Proger and David Ayman, 335
- Isaacs, Raphael, 990
- Jochim, Kenneth, 973
- Johnston, C G See Ravdin, I S
- Johnston, C G, Ravdin, I S, Riegel, C and Allison, C L, Studies on gallbladder function IX The amoncation content of bile from the normal and infected gallbladder, 67
- Johnston, Franklin D, 969, 993
- Johnston, M W See Newburgh, L H
- Johnston, S M See Hayman, J M, Jr
- Jolliffe, Norman See Chasis, Herbert
- Jonas, Leon, A note on cutaneous venous blood sugar difference in normal males and females and in thyroid disease, 139
- Jones, Edgar, 984
- Jones, T Duckett, 993
- Katz, L N, 973
- Keefer, Chester S See Myers, Walter K and Oppel, Theodore W
- Keefer, Chester S, Myers, Walter K and Oppel, Theodore W, Streptococcal agglutinins in patients with rheumatoid (atrophic) arthritis and acute rheumatic fever, 267
- Keith, Norman M, 964
- Khayat, George B, 992
- King, John T, 983
- Kirk, Esben, Amino nitrogen changes of the blood in nephritis, 1091
- Klumpp, Theodore G and Bowie, Morris A, Studies on gastric secretion, 1
- Kohn, Lawrence A, 971
- Korns, Horace M and Gunand, P H, Inequality of blood pressure in the brachial arteries, with especial reference to disease of the arch of the aorta, 143
- Kountz, William B, 970
- Kydd, David M See Peters, John P
- Kydd, David M, Salt and water in the treatment of diabetic acidosis, 1169
- Landis, Eugene M, 983

- Landis Eugene M and Gibbon John H Jr, The effects of temperature and of tissue pressure on the movement of fluid through the human capillary wall, 105
The effects of, alternate suction and pressure on blood flow to the lower extremities 925
- Lashmet F H 968
- Lavietes Paul H See Peters, John P
- Lawrence J A., 986
- Lawrence John S 984
- Lennox, W G 984
- Lerman J 973
- Lerman, J See Means, J H and Salter, W T
- Levine, Samuel A 979
- Liver bile after release of common duct obstruction I S Ravdin C G Johnston C. Riegel and S L Wright, 659
- Liver extract leukocytosis following the injection of John H Powers and William P Murphy with the assistance of Katharine Humphreys, 713
- Loeb Robert F See Atchley, Dana W
- Long, Perrin H, 965
- Long Perrin H, Herpetic pharyngitis and stomatitis 1119
- Long, Perrin H Bliss Eleanor A and Carpenter Harriet M Studies upon the nasal secretions I The cellular content of the nasal secretions in acute disease of the upper respiratory tract, 1127
- Lukens, Francis D W Attempts to produce experimental gastritis 181
- Lung volume. See pulmonary capacity
- Lung volume effect of laparotomy on Henry K. Beecher 651
- Lymph subcutaneous and blood proteins in dogs A A Weech E Goettsch and E B Reeves 1021
- Lyon James A 963
- Lyttle John D The Addis sediment count in normal children 87
The Addis sediment count in scarlet fever, 95
- Macy, John W., 964
- Magnesium metabolism in hyperparathyroidism Harold A Bulger and Florence Gausmann 1135
- Malignant tissue, effect of thyroxin on, Ovid O Meyer, Claire McTiernan and Joseph C Aub 723
- Man Evelyn B and Peters John P Permeability of capillaries to plasma lipoids 1031
- Manchester, Robert C, The diurnal rhythm in water and mineral exchange 995
- Margolies A 988
- McCann, Wm S See Hurtado Alberto
- McClugage H M 972
- McIntosh J F 967
- McLellan Lawrence L. See Thompson, Willard Owen
- McMillan, Thomas M, 970
- McNabb Paul E 963
- McQuarrie Irvine Bloor W R Husted Clara and Patterson H A The lipids of blood plasma in epilepsy I A statistical study of single determinations in 100 epileptic and 32 normal subjects 247
- McQuarrie Irvine, Husted Clara and Bloor W R The lipids of the blood plasma in epilepsy II Variations of lipids in relation to occurrence of seizures, 255
- McTiernan Claire. See Meyer Ovid O
- Means J H See Salter W T
- Means J H Lerman J and Salter, W T The role of thyroxin iodine and total organic iodine in the calorogenic action of whole thyroid gland, 683
- Medes Grace 968
- Merritt H Houston, 980
- Meyer Ovid O McTiernan, Claire and Aub Joseph C., The effect of thyroxin upon the metabolism of isolated normal and malignant tissue 723
- Michelsen Jost See Talbott, John H.
- Miller D G 965
- Miller David G Jr See Wells Herbert S
- Miller D K. 965
- Miller, D K. and Rhoads, C P, The effect of splenic contraction on the formed elements of the blood in a case of anemia and splenomegaly, 1009

- Mineral and water exchange, diurnal rhythm in, Robert C Manchester, 995
- Moen, Johannes K and Reimann, Hobart A, Plasma protein changes and suspension stability of the blood in lobar pneumonia, 589
- Morgan, Hugh J, 984
- Morton, John J, 988
- Muntwyler, Edward, Way, Charles T, Binns, Dorothy and Myers, Victor C, Plasma proteins and plasma colloid osmotic pressure in pathological conditions with special reference to the occurrence of edema, 495
- Murphy, William P, 985
- Murphy, William P See Powers, John P
- Myers, Victor C See Muntwyler, Edward
- Myers, Walter K See Keefer, Chester S and Oppel, Theodore W
- Myers, Walter K, Keefer, Chester S and Oppel, Theodore W, Skin Reactions to nucleoprotein of streptococcus scarlatinae in patients with rheumatoid arthritis and rheumatic fever, 279
- Nasal secretions, cellular content of, in acute disease of upper respiratory tract Perrin H Long, Eleanor A Bliss and Harriett M Carpenter, 1127
- Nephritis, amino nitrogen changes of the blood in, Esben Kirk, 1091
- Newburgh, L H, 968
- Newburgh, L H, Wiley, F H and Johnston, M W, A note on the calculation of water exchange, 1151
- Nicholls, Edith E See Stainsby, Wendell J
- Nicholls, Edith E and Stainsby, Wendell J, Further studies on the agglutination reaction in chronic arthritis, 505
- Oppel, Theodore W See Keefer, Chester S and Myers, Walter K
- Oppel, Theodore W, Myers, Walter K and Keefer, Chester S, The sedimentation rate of the red blood cells in various types of arthritis, 291
- Orthopnea, cisternal pressure in, William Groce Harrison, Jr, 1075
- Osterberg, Arnold E See Vanzant, Frances R
- Osterberg, Arnold E, Vanzant, Frances R. and Alvarez, Walter C, Studies of gastric pepsin I Methods of measurement and factors which influence it, 551
- Padget, Paul, 975
- Page, Irvine H, The action of certain diuretics on the function of the kidney as measured by the urea clearance test, 737
- Palmer, Robert Sterling, 972
- Pancreatic enzymes in pernicious anemia Paul J Fouts and L G Zervas, 519
- Patterson, H A See McQuarrie, Irvine Paul, John R, 986
- Payne, Warren See Spies, Tom Douglas
- Payne, Sheldon A and Perlzweig, William A, Cystine content of finger nails in pellagra, 899
- Pellagra and pernicious anemia Tom Douglas Spies and Warren Payne, 229
- Pellagra Cystine content of finger nails Sheldon A Payne and William A Perlzweig, 899
- Penick, Rawley M, 975
- Perlzweig, William A See Payne, Sheldon A
- Peters, John P See Man, Evelyn B
- Peters, John P and Laviates, Paul H, The nature of "preformed water," 695
- Peters, John P, Kydd, David M and Laviates, Paul H, A note on the calculation of water exchange, 689
- Peters, John P, Kydd, David M and Eisenman, Anna J, Serum proteins in diabetic acidosis, 355
- Peters, John P, Kydd, David M, Eisenman, Anna J and Hald, Pauline M, The nature of diabetic acidosis, 377
- Phatak, Nilkanth M See Smyth, Francis Scott
- Phillips, Francis J, 963
- Pneumonia, hemolytic streptococcus serum treatment of, Harold L Amoss and Erle B Craven, Jr, 885
- Pneumonia, lobar, experimental I Method of production and course of

- disease. Edward E Terrell Oswald
H Robertson and Lowell T Coggeshall 393
- Pneumonia lobar, experimental II Pathology Oswald H Robertson Lowell T Coggeshall and Edward E Terrell, 433
- Pneumonia lobar, experimental III Pathogenesis Oswald H Robertson Lowell T Coggeshall and Edward E Terrell 467
- Pneumonia lobar plasma protein changes in and suspension stability Johannes K. Moen and Hobart A Reimann 589
- Pollard W Scott The blood in cases of unexplained gastric anacidity 599
- Post Wilber, E See Hoffman William S
- Powers John H 985
- Powers, John H and Murphy William P with the assistance of Katharine Humphreys Leukocytosis following the intramuscular injection of liver extract 713
- Proceedings of the twenty fifth annual meeting of the American Society for Clinical Investigation held in Washington, D C. May 8 1933 963
- Proger Samuel H and Ayman David, Hyperventilation in arteriolar hypertension 335
- Proskourtakoff A 981
- Pulmonary capacity I Normal values Alberto Hurtado and Charles Boller 793
- Pulmonary capacity II Correlation of physical and radiological measurements Alberto Hurtado and Walter W Fray 807
- Pulmonary capacity III Change of body posture. Alberto Hurtado and Walter W Fray 825
- Pulmonary capacity IV In pulmonary emphysema and pneumoconiosis Alberto Hurtado Walter W Fray and Wm S McCann, 833
- Putnam, Tracy J 986
- Ravdin I S, Johnston, C G Riegel C. and Wright, S L. A study of human liver bile after release of common duct obstruction, 659
- Ravdin I S See Johnston, C G
- Ray George B, Thomas C. I and Strong, J E, The oxygenation of concentrated versus normal bloods 1051
- Reeves E. B See Weech A. A
- Reimann, Hobart A, 968
- Reimann Hobart A See Moen Johannes K
- Renal function action of diuretics measured by urea clearance test Irvine H Page 737
- Renal function action of phlorizin on excretion of glucose, xylose, sucrose, creatinine and urea. Herbert Chasis Norman Jolliffe and Homer W Smith, 1083
- Renal function, comparison of creatinine and urea clearance tests J M Hayman, Jr J A. Halsted and L E Seyler 861
- Renal function effect of salyrgan on water content of plasma. Henry Lenzen Schmitz 741
- Renal function effects on urea clearance of changes in protein and salt of diet Cuthbert L Cope 567
- Renal function rate of excretion of urine in different amounts of renal tissue David A Rytand 1153
- Renal function relation of creatinine and urea clearance tests and the number of glomeruli J M Hayman Jr and S M Johnston, 877
- Respiration collateral C M Van Allen and Y C. Soo 171
- Respiration, effect of laparotomy on Henry K Beecher 639
- Reznikoff, Paul The treatment of agranulocytosis with adenine sulphate 45
- Rheumatic fever, agglutinating properties of, exudates from, Charles H. Hitchcock and Homer F Swift 673
- Rheumatoid arthritis and acute rheumatic fever, streptococcal agglutinins in Chester S Keefer, Walter K. Myers and Theodore W Oppel 267
- Rheumatoid arthritis erythrocytic sedimentation test in, Wendell J Stansby and Edith E. Nicholls 1041
- Rheumatoid arthritis in rheumatic fever skin reactions to nucleoprotein and

- streptococcal agglutinins in, Walter K. Myers, Chester S. Keefer and Theodore W. Oppel, 279
- Rhoads, C. P. See Miller, D. K.
- Richards, Dickinson W., Jr. See Atchley, Dana W.
- Richardson, H. B., 966
- Richardson, Russell, Immunity in diabetes influence of diabetes on the development of antibacterial properties in the blood, 1143
- Riecker, Herman H., 986
- Riegel, C. See Johnston, C. G. and Ravdin, I. S.
- Rivers, Andrew B. See Vanzant, Frances R.
- Robertson, Oswald H. See Terrell, Edward E. and Wallace, Helene
- Robertson, Oswald H., Coggeshall, Lowell T. and Terrell, Edward E., Experimental pneumococcus lobar pneumonia in the dog II Pathology, 433
- Experimental pneumococcus lobar pneumonia in the dog III Pathogenesis, 467
- Root, Howard F., 987
- Rosenow, Edward C., 987
- Roth, Grace M., 977
- Rothschild, Herbert, 969
- Ruffin, Julian M., 963
- Rytand, David A., The effect of digitalis on the venous pressure of normal individuals, 847
- The rate of excretion of urine in subjects with different amounts of renal tissue, 1153
- Salter, W. T., 973
- Salter, W. T. See Means, J. H.
- Salter, W. T., Lerman, J. and Means, J. H., The calorogenic action of thyroxin polypeptide, 327
- Saunders, George M., 971
- Schmitz, Henry Lenzen, Studies on the action of diuretics II The effect of salyrgan upon the water content of the plasma as measured by the refractive index, 741
- Scott, W. J. Merle, 988
- Seyler, L. E. See Hayman, J. M., Jr.
- Sheard, Charles See Craig, Winchell McK.
- Shorr, E., 966
- Short, Charles L., 967
- Sly, Grace E., 976
- Smith, David T., 963
- Smith, Homer W. See Chasis, Herbert
- Smyth, Francis Scott, Callahan, William and Phatak, Nilkanth M., Studies in so-called water intoxication, 55
- Snelling, C. E. See Weech, A. A.
- Snyder, Maurice, 980
- Soo, Y. C. See Van Allen, C. M.
- Spies, Tom D., 991
- Spies, Tom Douglas and Payne, Warren, A study of the etiological relationship between pellagra and pernicious anemia, 229
- Splenic contraction, effect of, on the blood in anemia and splenomegaly D. K. Miller and C. P. Rhoads, 1009
- Stainsby, Wendell J. See Nicholls, Edith E.
- Stainsby, Wendell J. and Nicholls, Edith E., The clinical significance of the erythrocytic sedimentation test in rheumatoid arthritis, 1041
- Starr, Isaac, Jr., 988
- Starr, Isaac, Jr., Collins, Leon H., Jr. and Wood, Francis Clark, Studies of the basal work and output of the heart in clinical conditions, 13
- Stephens, Doran J., 984
- Stewart, Harold J., 989
- Stewart, John D., 978
- Stomatitis, herpetic Perrin H. Long, 1119
- Strang, J. M., 972
- Strauss, Maurice B., Anemia of infancy from maternal iron deficiency in pregnancy, 345
- Strayhorn, W. David, Jr., 977
- Strock, Moses S., 974
- Strong, J. E. See Ray, George B.
- Sturgis, Cyrus C., 990
- Sunderman, F. William, 994
- Swift, Homer F. See Hitchcock, Charles H.
- Sympathetic ganglionectomy under general anesthesia thermal changes Winchell McK. Craig, Bayard T. Horton and Charles Sheard, 573
- Talbott, John H., 978
- Talbott, John H. and Michelsen, Jost.

- Heat cramps A clinical and chemical study 533
- Taylor Samuel G III 990
- Terrell Edward E See Robertson Oswald H
- Terrell Edward E Robertson Oswald H and Coggeshall Lowell T Experimental pneumococcus lobar pneumonia in the dog I Method of production and course of the disease 393
- Thomas, C. I See Ray George B
- Thomas Clarence S 984
- Thompson, Phebe K., 990
- Thompson Phebe K See Thompson Willard Owen
- Thompson, Willard O 990
- Thompson Willard Owen McLellan Lawrence L Thompson Phebe, K. and Dickie Lois F N The rates of utilization of thyroxine and of desiccated thyroid in man The relation between iodine in desiccated thyroid and in thyroxine 235
- Thyroid, calorogenic action of role of thyroxin iodine and total organic iodine. J H Means, J Lerman and W T Salter, 683
- Thyroid disease cutaneous venous blood sugar difference. Leon Jonas 139
- Thyroid relation between iodine in desiccated thyroid and thyroxine Wilard Owen Thompson Lawrence L McLellan Phebe K. Thompson and Lois F N Dickie 235
- Thyroxin effect of on metabolism of isolated and malignant tissues Ovid O Meyer Claire McTiernan and Joseph C Aub 723
- Thyroxin polypeptide calorogenic action of W T Salter J Lerman and J H Means 327
- Tillett, William S 991
- Trask James D 969
- Turner Kenneth B, 992
- Turner, Thomas B, 971
- Urine Addis sediment count in normal children. John D Lyttle, 87
- Urine Addis sediment count in scarlet fever John D Lyttle 95
- Van Allen C. M and Soo Y C. Collateral respiration Spontaneous re-inflation of an atelectatic pulmonary lobule by collateral respiration, 171
- Vanzant, Frances R See Osterberg Arnold E
- Vanzant Frances R., Osterberg Arnold E, Alvarez Walter C and Rivers Andrew B, Studies of gastric pepsin I Secretion of pepsin in cases of duodenal ulcer and pseudo ulcer 557
- Venous pressure, effect of digitalis David A Rytand 847
- Volk, Marie C. 975
- Wallace E W See Wallace Helene
- Wallace, Helene, Wallace E W and Robertson O H The production of experimental Plaut Vincent's angina in the dog, 909
- Warren Stafford L., 971
- Water and mineral exchange diurnal rhythm in Robert C Manchester, 995
- Water exchange calculation of John P Peters David M Kydd and Paul H Laviates 689
- Water exchange calculation of L H Newburgh F H Wiley and M W Johnston 1151
- Water exchange nature of preformed water John P Peters and Paul H Laviates 695
- Water intoxication. Francis Scott Smyth William Callahan and Nilkanth M Phatak 55
- Way Charles T See Muntwyler Edward
- Weech A. A Goettsch E and Reeves E B The effect of serum transfusion on the plasma protein depletion associated with nutritional edema in dogs 217
- The proteins of blood and subcutaneous lymph in dogs 1021
- Weech A. A., Snelling C E and Goettsch, E The relation between plasma protein content plasma specific gravity and edema in dogs maintained on a protein inadequate diet and in dogs rendered edematous by plasmapheresis 193
- Weiss Soma 992
- Wells H S 965
- Wells Herbert S Youmans John B and Miller, David G Jr, A formula and

- nomogram for the estimation of the osmotic pressure of colloids from the albumin and total protein concentrations of human blood sera, 1103
- White, Paul D, 993
- Wiley, F H See Newburgh, L H
- Wilkins, Walter E and Cullen, Glenn E, Electrolytes in human tissue III A comparison of normal hearts with hearts showing congestive heart failure, 1063
- Williams, Enmon, 994
- Wilson, Frank N, 969, 993
- Winkler, Alexander W, 972
- Wolferth, Charles C, 970
- Wolff, H G, 966
- Wood, Francis Clark, 970
- Wood, Francis Clark See Starr, Isaac, Jr
- Wood, J Edwin, Jr, 993
- Wright, S L See Ravdin, I S
- Yannet, Herman and Darrow, Daniel C, Physiological disturbances during experimental diphtheritic intoxication I Blood sugar, lactic acid and non-protein and amino-acid nitrogen, 767
- Physiological disturbances during experimental diphtheritic intoxication II Hepatic glycogenesis and glycogen concentration of cardiac and skeletal muscle, 779
- Yanner, Herman and Goldfarb, Walter, Physiological disturbances during experimental diphtheritic intoxication III Respiratory quotients and metabolic rate, 787
- Yater, Wallace M, 963
- Youmans, John B, 965
- Youmans, John B See Wells, Herbert S
- Zerfas, L G See Helmer, O M

CONTENTS

NUMBER 1, JANUARY, 1933

Studies on Gastric Secretion THEODORE G KLUMPP AND MORRIS A BOWIE	1
Studies of the Basal Work and Output of the Heart in Clinical Conditions ISAAC STARR, JR, LEON H COLLINS, JR AND FRANCIS CLARK WOOD	13
The Treatment of Agranulocytosis with Adenine Sulphate PAUL REZNIKOFF	45
Studies in So Called Water Intoxication FRANCIS SCOTT SMYTH, WILLIAM CALLAHAN AND NILKANTH M PHATAK	55
Studies on Gallbladder Function IX The Anion Cation Content of Bile from the Normal and Infected Galbladder C G JOHNSTON, I S RAVDIN, C RIEGEL AND C L ALLISON	67
The Unavoidable Error in the Differential Count of the Leukocytes of the Blood C W BARNETT	77
The Addis Sediment Count in Normal Children JOHN D LYTTLE	87
The Addis Sediment Count in Scarlet Fever JOHN D LYTTLE	95
The Effects of Temperature and of Tissue Pressure on the Movement of Fluid through the Human Capillary Wall EUGENE M LANDIS AND JOHN H GIBBON, JR	105
A Note on Cutaneous Venous Blood Sugar Difference in Normal Males and Females and in Thyroid Disease LEON JONAS	139
Inequality of Blood Pressure in the Brachial Arteries, with Especial Reference to Disease of the Arch of the Aorta HORACE M KORN AND P H GUINAND	143
Gastric Secretion in Fever and Infectious Diseases HSIAO-CH' IEN CHANG	155
Collateral Respiration Spontaneous Reinflation of an Atelectatic Pulmonary Lobule by Collateral Respiration C M VAN ALLEN AND Y C Soo	171
Attempts to Produce Experimental Gastritis FRANCIS D W LUKENS	181
The Relation Between Plasma Protein Content, Plasma Specific Gravity and Edema in Dogs Maintained on a Protein Inadequate Diet and in Dogs Rendered Edematous by Plasmapheresis A A WEECH, C E SNELLING AND E GOETTSCHE	193
The Effect of Serum Transfusion on the Plasma Protein Depletion Associated with Nutritional Edema in Dogs A A WEECH, E GOETTSCHE AND E B REEVES	217

A Study of the Etiological Relationship Between Pellagra and Pernicious Anemia	TOM DOUGLAS SPIES AND WARREN PAYNE	229
The Rate of Utilization of Thyroxine and of Desiccated Thyroid in Man	The Relation Between the Iodine in Desiccated Thyroid and in Thyroxine	
	WILLARD OWEN THOMPSON, LAWRENCE L McLELLAN, PHEBE K THOMPSON AND LOIS F N DICKIE	235

NUMBER 2, MARCH, 1933

The Lipids of the Blood Plasma in Epilepsy I A Statistical Study of Single Determinations in 100 Epileptic and 32 "Normal" Subjects	IRVINE McQUARRIE, W R BLOOR, CLARA HUSTED AND H A PATTERSON	247
The Lipids of the Blood Plasma in Epilepsy II Variations of Lipids in Relation to Occurrence of Seizures	IRVINE McQUARRIE, CLARA HUSTED AND W R BLOOR	255
Streptococcal Agglutinins in Patients with Rheumatoid (Atrophic) Arthritis and Acute Rheumatic Fever	CHESTER S KEEFER, WALTER K MYERS AND THEODORE W OPPEL	267
Skin Reactions to Nucleoprotein of Streptococcus Scarlatinae in Patients with Rheumatoid Arthritis and Rheumatic Fever	WALTER K MYERS, CHESTER S KEEFER AND THEODORE W OPPEL	279
The Sedimentation Rate of the Red Blood Cells in Various Types of Arthritis	THEODORE W OPPEL, WALTER K MYERS AND CHESTER S KEEFER	291
On Diabetic Acidosis A Detailed Study of Electrolyte Balances Following the Withdrawal and Reestablishment of Insulin Therapy	DANA W ATCHLEY, ROBERT F LOEB, DICKINSON W RICHARDS, JR, ETHEL M BENEDICT AND MARY E DRISCOLL	297
The Calorigenic Action of Thyroxin Polypeptide	W T SALTER, J LERMAN AND J H MEANS	327
Hyperventilation in Arteriolar Hypertension	SAMUEL H PROGER AND DAVID AYMAN	335
Anemia of Infancy from Maternal Iron Deficiency in Pregnancy	MAURICE B STRAUSS	345
Serum Proteins in Diabetic Acidosis	JOHN P PETERS, DAVID M KYDD AND ANNA J EISENMAN	355
The Nature of Diabetic Acidosis	JOHN P PETERS, DAVID M KYDD, ANNA J EISENMAN AND PAULINE M HALD	377
Experimental Pneumococcus Lobar Pneumonia in the Dog I Method of Production and Courses of the Disease	EDWARD E TERRELL, OSWALD H ROBERTSON AND LOWELL T COGGESHALL	393
Experimental Pneumococcus Lobar Pneumonia in the Dog II Pa-		

thology	OSWALD H ROBERTSON, LOWELL T COGGESHALL AND EDWARD E TERRELL	433
Experimental Pneumococcus Lobar Pneumonia in the Dog	III Pathogenesis OSWALD H ROBERTSON, LOWELL T COGGESHALL AND EDWARD E TERRELL	467
Plasma Protein and Plasma Colloid Osmotic Pressure in Pathological Conditions with Special Reference to the Occurrence of Edema	EDWARD MUNTWYLER, CHARLES T WAY, DOROTHY BINNS AND VICTOR C MYERS	495

NUMBER 3, MAY, 1933

Further Studies on the Agglutination Reaction in Chronic Arthritis	EDITH E NICHOLLS AND WENDELL J STAINSBY	505
Gastro Intestinal Studies II Pancreatic Enzymes in Pernicious Anemia	O M HELMER, PAUL J FOUTS AND L G ZERFAS	519
Heat Cramps A Clinical and Chemical Study	JOHN H TALBOTT AND JOST MICHELSEN	533
Studies of Gastric Pepsin I Methods of Measurement and Factors which Influence It	ARNOLD E OSTERBERG, FRANCES R VANZANT AND WALTER C ALVAREZ	551
Studies of Gastric Pepsin II Secretion of Pepsin in Cases of Duodenal Ulcer and Pseudo ulcer	FRANCES R VANZANT, ARNOLD E OSTERBERG, WALTER C ALVAREZ AND ANDREW B RIVERS	557
Studies of Urea Excretion VIII The Effects on the Urea Clearance of Changes in Protein and Salt Contents of the Diet	CUTHBERT L COPE	567
Thermal Changes in Peripheral Vascular Disease During Sympathetic Ganglionectomy Under General Anesthesia	WINCHELL MCK CRAIG BAYARD T HORTON AND CHARLES SHEARD	573
Glycolysis in the Blood of Patients with Pernicious Anemia	S MILTON GOLDHAMER	583
Plasma Protein Changes and Suspension Stability of the Blood in Lobar Pneumonia	JOHANNES K MOEN AND HOBART A. REIMANN	589
The Blood in Cases of Unexplained Gastric Anacidity	W SCOTT POLLAND	599

NUMBER 4, JULY, 1933

The Influence of Mineral Metabolism upon Nephrotic Edema.	WILLIAM S HOFFMAN AND WILBER E POST	613
The Measured Effect of Laparotomy on the Respiration	HENRY K BEECHER	639

Effect of Laparotomy on Lung Volume Demonstration of a New Type of Pulmonary Collapse HENRY K BEECHER	651
A Study of Human Liver Bile after Release of Common Duct Obstruction I S RAVDIN, C G JOHNSTON, C RIEGEL AND S L WRIGHT	659
The Agglutinating Properties of Exudates from Patients with Rheumatic Fever CHARLES H HITCHCOCK AND HOMER F SWIFT	673
The Rôle of Thyroxin Iodine and Total Organic Iodine in the Calorigenic Action of Whole Thyroid Gland J H MEANS, J LERMAN AND W T SALTER	683
A Note on the Calculation of Water Exchange JOHN P PETERS, DAVID M KYDD AND PAUL H LAVIETES	689
The Nature of "Preformed Water" JOHN P PETERS AND PAUL H LAVIETES	695
Leukocytosis Following the Intramuscular Injection of Liver Extract JOHN H POWERS AND WILLIAM P MURPHY WITH THE ASSISTANCE OF KATHARINE HUMPHREYS	713
The Effect of Thyroxin upon the Metabolism of Isolated Normal and Malignant Tissue OVID O MEYER, CLAIRE McTIERNAN AND JOSEPH C AUB	723
The Action of Certain Diuretics on the Function of the Kidney as Measured by the Urea Clearance Test IRVINE H PAGE	737

NUMBER 5, SEPTEMBER, 1933

Studies on the Action of Diuretics II The Effect of Salyrgan upon the Water Content of the Plasma as Measured by the Refractive Index HENRY LENZEN SCHMITZ	741
Studies in Congestive Heart Failure XXIII A Critical Study of Methods for Determining the Cardiac Output in Patients with Cardiac Disease ARTHUR GROLLMAN, BEN FRIEDMAN, GURNEY CLARK AND T R HARRISON	751
Physiological Disturbances During Experimental Diphtheritic Intoxication I Blood Sugar, Lactic Acid and Non-Protein and Amino-Acid Nitrogen HERMAN YANNET AND DANIEL C DARROW	767
Physiological Disturbances During Experimental Diphtheritic Intoxication II Hepatic Glycogenesis and Glycogen Concentration of Cardiac and Skeletal Muscle HERMAN YANNET AND DANIEL C DARROW	779
Physiological Disturbances During Experimental Diphtheritic Intoxication III Respiratory Quotients and Metabolic Rate HERMAN YANNET AND WALTER GOLDFARB	787

Studies of Total Pulmonary Capacity and Its Subdivisions I Normal, Absolute and Relative Values ALBERTO HURTADO AND CHARLES BOLLER	793
Studies of Total Pulmonary Capacity and Its Subdivisions II Correlation with Physical and Radiological Measurements ALBERTO HURTADO AND WALTER W FRAY	807
Studies of Total Pulmonary Capacity and Its Subdivisions III Changes with Body Posture ALBERTO HURTADO AND WALTER W FRAY	825
Studies of Total Pulmonary Capacity and Its Subdivisions IV Preliminary Observations on Cases of Pulmonary Emphysema and of Pneumoconiosis ALBERTO HURTADO, WALTER W FRAY AND WM S MCCANN	833
The Effect of Digitalis on the Venous Pressure of Normal Individuals DAVID A RYTAND	847
A Comparison of the Creatinine and Urea Clearance Tests of Kidney Function J M HAYMAN, JR, J A HALSTED AND L E SEYLER	861
Experiments on the Relation of Creatinine and Urea Clearance Tests of Kidney Function and the Number of Glomeruli in the Human Kidney Obtained at Autopsy J M HAYMAN, JR, AND S M JOHNSTON	877
Serum Treatment of Hemolytic Streptococcus Pneumonia HAROLD L AMOSS AND ERLE B CRAVEN, JR	885
Cystine Content of Finger Nails in Pellagra SHELDON A PAYNE AND WILLIAM A PERLZWEIG	899
The Production of Experimental Plaut-Vincent's Angina in the Dog HELENE WALLACE, E W WALLACE AND O H ROBERTSON	909
The Effects of Alternate Suction and Pressure on Blood Flow to the Lower Extremities EUGENE M LANDIS AND JOHN H GIBBON, JR	925
Proceedings of the Twenty fifth Annual Meeting of the American Society for Clinical Investigation held in Washington, D C, May 8 1933	963

NUMBER 6 NOVEMBER, 1933

The Diurnal Rhythm in Water and Mineral Exchange ROBERT C MANCHESTER	995
The Effect of Splenic Contraction on the Formed Elements of the Blood in a Case of Anemia and Splenomegaly D K MILLER AND C P RHOADS	1009
The Proteins of Blood and Subcutaneous Lymph in Dogs A A WEECH, E GOETTSCH AND E B REEVES	1021

Permeability of Capillaries to Plasma Lipoids	EVELYN B MAN AND JOHN P PETERS	1031
The Clinical Significance of the Erythrocytic Sedimentation Test in Rheumatoid Arthritis	WENDELL J STAINSBY AND EDITH E NICHOLLS	1041
The Oxygenation of Concentrated Versus Normal Bloods	GEORGE P RAY, C I THOMAS AND J E STRONG	1051
Electrolytes in Human Tissue III A Comparison of Normal Hearts with Hearts Showing Congestive Heart Failure	WALTER E WILKINS AND GLENN E CULLEN	
The Cisternal Pressure in Congestive Heart Failure and Its Bearing on Orthopnea	WILLIAM GROCE HARRISON, JR	1075
The Action of Phlorizin on the Excretion of Glucose, Xylose, Sucrose, Creatinine and Urea by Man	HERBERT CHASIS, NORMAN JOLLIFFE AND HOMER W SMITH	1083
Amino Nitrogen Changes of the Blood in Nephritis	ESBEN KIRK	1091
A Formula and Nomogram for the Estimation of the Osmotic Pressure of Colloids from the Albumin and Total Protein Concentrations of Human Blood Sera	HERBERT S WELLS, JOHN B YOUNG, AND DAVID G MILLER, JR	1103
Herpetic Pharyngitis and Stomatitis A Report of Three Cases	PERRIN H LONG	1119
Studies Upon the Nasal Secretions I The Cellular Content of the Nasal Secretions in Acute Disease of the Respiratory Tract	PERRIN H LONG, ELEANOR A BLISS AND HARRIETT M CARPENTER	1127
Magnesium Metabolism in Hyperparathyroidism	HAROLD A BULGER AND FLORENCE GAUSMANN	1135
Immunity in Diabetes Influence of Diabetes on the Development of Antibacterial Properties in the Blood	RUSSELL RICHARDSON	1143
A Note on the Calculation of Water Exchange	L H NEWBURGH, F H WILEY AND M W JOHNSTON	1151
The Rate of Excretion of Urine in Subjects with Different Amounts of Renal Tissue	DAVID A RYTAND	1153
Salt and Water in the Treatment of Diabetic Acidosis	DAVID M KYDD	1169
Index to Volume XII		1185

TABLE I
Data on blood

Patient	Day after onset	Plasma									Blood sedimentation time
		Total protein	Albumin	Total globulin	Fibrinogen	Euglobulin	Pseudo-globulin I	Pseudo-globulin II	Albumin/Globulin ratio	Viscosity	
		grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc	grams per 100 cc			minutes
Case I P G	2	7.26	3.61	3.65	0.79	0.94	1.17	0.75	0.99		115
	6	6.41	3.49	2.92	1.06	0.20	1.06	0.59	1.19		30
	14	6.04	3.35	2.69	0.79	0.19	0.98	0.73	1.24	1.9	97
	36	6.68	4.13	2.55	0.49	0.47	0.91	0.69	1.61	1.5	180+
Case III A K	5	5.61	2.84	2.77	0.27	0.33	0.99	1.20	1.03	2.1	18
	30	6.76	3.07	3.69	0.53	0.38	1.95	0.84	0.81	1.6	155
Case IV F F	11	5.99	2.18	3.81	0.74	0.66	1.31	1.10	0.57	1.8	7
	64	6.56	3.73	2.83	0.61	0.52	1.13	0.59	1.31	1.6	180+
Case VI J B	7	6.55	3.14	3.41	0.59	0.75	1.30	0.77	0.91	2.0	16
	48	6.35	3.12	3.23	0.31	0.38	1.98	0.57	0.96	1.5	180+
Normal amounts		6.67	4.07	2.59	0.29	0.68	0.94	0.68	1.57	1.7	180+

Case II M B Female, aged 37 Type III pneumococcus infection, right lower lobe Approximately 18 hours after the initial chill the amount of fibrinogen had increased to 0.93 gram, the maximum observed in this patient. The minimum sedimentation time, however, was not reached until the sixth day when the viscosity was greatest, the fibrinogen diminished but the total globulin increased slightly. During convalescence the amount of fibrinogen and globulin decreased, the viscosity diminished and the sedimentation time increased until normal values were found on the thirty-ninth day. See Figure 1.

Case III A K Male, aged 28 Type III pneumococcus infection, left lower lobe. In this patient the fibrinogen and total globulin were practically normal in amount on the fifth day of illness, yet the sedimentation time was short (18 minutes) and the viscosity 2.1. During convalescence, on the thirtieth day, both the amount of fibrinogen and total globulin was increased, the viscosity decreased and the sedimentation time was nearly normal. See Table I. Observations in this case corroborate the findings of others who showed that increase in sedimentation time did not always parallel decrease in globulins.

Case IV F F Male, aged 37 Pneumococcus group IV infection, right upper and lower lobes. The amount of globulin and of fibrinogen was increased together with an increased viscosity and diminished sedimentation time as shown in Table I. On the sixty-fourth day, the viscosity and sedimentation time were normal but the amount of fibrinogen was still increased to 0.61 gram.

Case V S S Male, aged 29 Group IV pneumococcus infection, four lobes involved. Although the fibrinogen content increased markedly between